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QUARTERLY JOURNAL OF THE FLORIDA ACADEMY OF SCIENCES

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Biological Sciences

DISTRIBUTION OF THREE COMMON SPECIES OF DAMSELFISH ON PATCH REEFS WITHIN THE DRY TORTUGAS NATIONAL PARK, FLORIDA

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ABSTRACT: *During the late 1970s and '80s an extreme cold front followed by an outbreak of White Band Disease destroyed most of the staghorn reef formations in Dry Tortugas National Park (DTNP), Florida, and dramatically altered the reef structure. We studied damselfish assemblages on patch reefs off Loggerhead Key in DTNP during April 2002. We found that dusky (*Pomacentrus fuscus*), cocoa, (*Pomacentrus variabilis*), and yellowtail (*Microspathodon chrysurus*) damselfishes were common on patch reefs and had respective densities of 0.33, 0.32, and 0.06 fish/m^2. Cocoa damselfish was the most widely distributed species, possibly due to its ability to recruit to the reef as juveniles, and to colonize more demanding reef habitats. Spatial partitioning across reefs was observed among the three damselfishes with monospecific aggregations occurring on 55% of plots and multi-species aggregations on 45% of plots and, weak vertical partitioning patterns were only observed for dusky damselfish. Density, distribution, and vertical stratification of territorial damselfishes differed from pre-1976 data reported from Alligator and Looe Key reefs, Florida, suggesting that these fishes may adjust population assemblages to accommodate catastrophic alterations to their physical habitat due to events such as cold or disease.*

Key Words: damselfish distribution, Dry Tortugas National Park, Pomacentridae

DAMSELFISHES (family Pomacentridae) are common residents on coral reefs from south Florida throughout the Caribbean and into the Bahamas (Lieske and Myers, 1999). Of the 16 species known to occur throughout North America (AFS, 1991), fewer than one-half are gregarious schooling fishes; the remainder are solitary species that generally confine their movements to well-defined territories, which they vigorously defend (Sweatman and St. John, 1990). Of the solitary damselfishes, the dusky damselfish (*Pomacentrus fuscus*), cocoa damselfish (*Pomacentrus variabilis*), and yellowtail damselfish (*Microspathodon chrysurus*), are the dominant species found in the northern Caribbean and Florida Keys (Emery, 1973). All three damselfish species are sympatrically distributed among massive coral patches as well as branching coral formations on the reef's outer fringe (Sweatman and St. John, 1990). Typically, more than one of these principal species can be found inhabiting a given patch or fringe reef area, leading to interspecific competition for space and resources within the reef.

Dusky, cocoa, and yellowtail damselfishes may effectively partition reef habitats through subtle distribution shifts among reef types and differences in vertical stratification patterns. Robertson (1984) reports that while adult yellowtail damselfish can be found among patch reef boulder corals, the highest densities occur among small clumps of staghorn (*Acropora cervicornis*), elkhorn (*A. palmata*), and fire (*Millepora* spp.) corals growing on the tops of shallow patch reefs. Conversely, adult dusky damselfish show a stronger preference for the shallow upper regions of branching coral and coralline rock formations than for patch reef habitat. Juveniles of both species may prefer other habitats, as they are not commonly observed on either reef type (Emery, 1973; Robertson, 1984). Cocoa damselfish on the other hand, do not appear to prefer any specific habitat type (McGehee, 1995), but rather are ubiquitous as both adults and juveniles from shallow patch reef tops to deeper waters along reef margins (Emery, 1973).

Reef structure and conformation clearly play an important role in shaping fish assemblages; as a result, events such as catastrophic weather or coral disease outbreaks may disrupt fish distribution patterns by radically altering reef cover and zonation. During the winter of 1976–77, for example, a devastating cold event along the Florida reef tract resulted in the decrease of many coral species and dramatically altered reef structure and fish diversity (Bohnsack, 1983). The Dry Tortugas National Park (DTNP) was especially hard hit with ninety-six percent of corals within two meters of the surface eliminated as a result of the extreme low temperatures (Porter et al., 1982). Staghorn coral, which prior to the cold front made up over half of all live coral in the area, suffered the most extensive mortality, whereas other coral species including round starlet (*Siderastrea siderea*), thin finger coral (*Porites furcata*), and rough cactus coral (*Mycetophyllia ferox*) received only minor damage (Davis, 1982; Porter et al., 1982; Roberts and Rouse, 1982). Habitat loss was further exacerbated during the late 1980s when an outbreak of White Band Disease nearly wiped out remaining staghorn reefs throughout the Caribbean (Aronson and Precht, 1997), including DTNP. Although many reef-dependent fish populations in the area were altered by the massive mortality and subsequent collapse of the staghorn reefs (Bohnsack, 1983), no study to date has assessed the post-impact status of the territorial damselfish assemblage in DTNP.

Our research evaluates patch reef assemblages of dusky, cocoa, and yellowtail damselfishes in DTNP, Florida. Specific objectives of our research were to estimate areal densities, identify differences in distribution, and compare vertical stratification of dusky, cocoa, and yellowtail damselfish species from patch reef areas. Comparisons of our post-impact findings with similar data collected from patch reefs in the Florida Keys prior to the cold event and white band disease outbreak provide

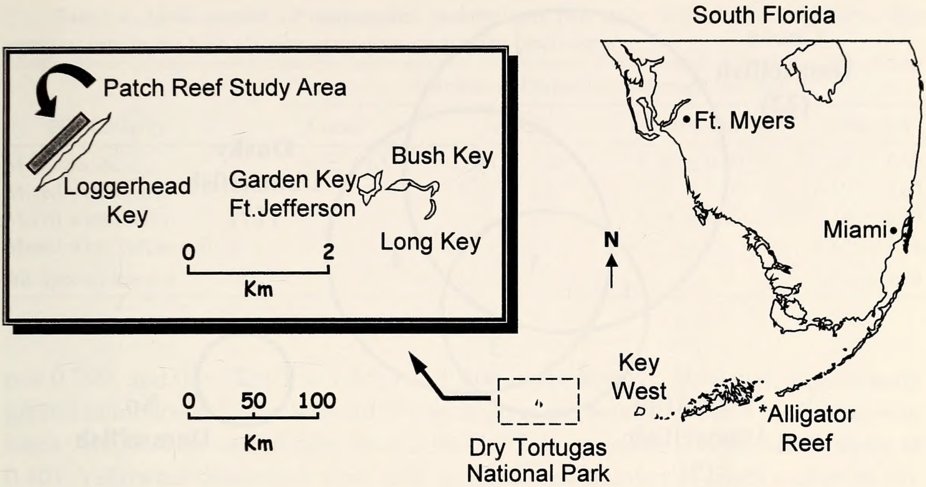

FIG. 1. Patch reef study site off Loggerhead Key, Dry Tortugas National Park, Florida, USA.

insights into how population assemblages of these species differ with varying reef structure and composition.

METHODS—*Sampling regimen*—Damselfish observations were made on patch reefs located between 30 and 70 meters from shore along the northeast side (24°38′39″N/ 82°57′7″W) of Loggerhead Key, DTNP (Fig. 1). Observations of abundance, position and life stages of damselfishes, as well as habitat characterizations were made on 33 individual patch reefs from 27 to 30 April 2002. Nearly equal numbers of observations were made between 0800 and 1200 EST (14) and 1300 to 1700 EST (19) so as to account for potential temporal changes in damselfish abundance. Temperature (±0.1°C), salinity (±1‰), and pH (±0.5 units) measurements were made at a location central to the patch reefs 1 m below the surface prior to each observation sequence. Observations on each patch reef were made in a 2 × 2 m plot, marked out just prior to the observational period, using lengths of twine and survey flags. For reefs smaller than 2 × 2 m, the observation plot contained the entire patch formation. Damselfishes typically defend territories of limited size; therefore, patch reefs smaller than 1 m^2 were not included in this study. For larger patch reefs, plots were placed either within the patch, or on its margin. Plots on large patch reefs were not randomly chosen, but were positioned such that observers had a clear field of view of the entire plot.

Teams of two observers snorkeling at each patch reef collected damselfish abundance, position, and life stage data during 40-minute periods. Data were recorded at seven discrete intervals as follows: during the first ten minutes of observation, no data were collected as fish were allowed to become accustomed to the observers' presence. In all cases, damselfishes appeared to resume normal milling and guarding behaviors within the allotted 10-minute acclimation period. Beginning at ten minutes, and at five-minute intervals thereafter, observer one recorded the number of each damselfish species present and identified each as adult or juvenile by its color phase. The second observer recorded the vertical location of each damselfish within the observation plot. Vertical location on the reef was defined as either bottom—a territory contacting the bottom substrate, middle—a territory with patch reef above and below, or top—a territory with patch reef below and open water above. This technique was repeated at each of the 33 patch reefs over the course of a four-day period.

Data analysis—We used parametric one-way block Analysis of Variance (ANOVA) on ranked data to assess intraspecific vertical stratification preferences, and to determine statistical relationships among species densities. Data were blocked on patch reefs (1-33). Where applicable, Student-Newman-Kuels multiple range tests (SNK MRT) were used to evaluate relationships between multiple means. The number

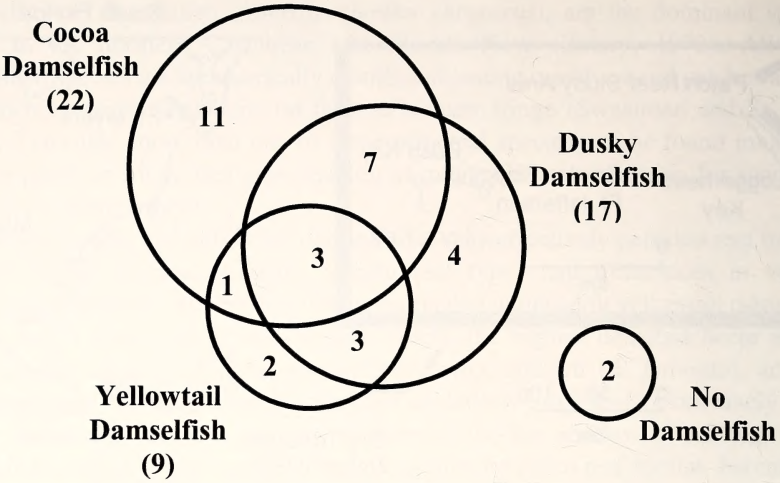

FIG. 2. Distribution of monospecific and conspecific observation plots, containing three damselfish species, adjacent to Loggerhead Key in Dry Tortugas National Park, Florida, USA.

of damselfish at each vertical position on any given plot was calculated as the mean value of all seven observations made at that plot. The average number of fish at each position for the entire study area was estimated as the grand mean of the individual plot values. Average plot density/m^2 for each damselfish species was calculated similarly. We evaluated differences between juvenile and adult cocoa damselfish average density/m^2 using Student's independent t-test (t-test). All statistical decisions were based on an alpha of 0.10.

RESULTS—Typical reef patches were comprised of approximately 15 to 30% live hermatypic boulder (*Montastrea*) and/or branching (*Acropora* and *Millepora*) corals interspersed with dead corals, soft corals, algae, sponges and coral rubble or sand. Patch reefs occurred at depths of 2 to 4 m depending on tidal stage, and ranged in area from approximately 0.25 to 100 m^2. The study site had temperature, salinity and pH ranges of 24.4 to 26.7°C, 35 to 38‰, and 8.2 to 8.4, respectively. Observation conditions were good with sunny to partly cloudy skies and water visibility of 10 to 15 m.

Damselfishes were seen on all but two of the 33 plots monitored; however, the three species were not equally distributed across patch reefs. Monospecific aggregations were seen in 17 plots (55%), while conspecific aggregations occurred in 14 plots (i.e., 45%). All three species of damselfishes occurred together on only three plots or 10% of the observations, whereas the remaining 11 conspecific aggregations (35%) contained only two damselfish species. Dusky/cocoa damselfish assemblages were most prevalent followed by dusky/yellowtail assemblages, and finally cocoa/yellowtail aggregations (Fig. 2). Interestingly, while damselfish distribution between plots was patchy, vertical stratification of fishes within patch reefs was only noted in dusky damselfish. No significant differences were found in the numbers of cocoa damselfish, or yellowtail damselfish observed at top, middle, or bottom reef positions (one-way block ANOVA on ranked data; df = 2,21; F = 0.24;

TABLE 1. Mean number of damselfishes observed per plot along with standard deviation. Plot category depicts the type of aggregation seen on specific patch reefs.

	Number of Damselfish Observed Per Plot			
Plot Category	Cocoa	Dusky	Yellow-tail	Total fish
Monospecific	1.90 ± 1.65	3.00 ± 1.40	1.18 ± 0.97	6.08 ± 1.57
Mixed with Cocoa		3.29 ± 1.43	3.28 ± 0.00	6.57 ± 1.03
Mixed with Dusky			5.15 ± 0.43	8.44 ± 1.37
Mixed with Yellow-tail				8.43 ± 1.74
All Species Present				5.61 ± 2.03

p = 0.790, and df = 2,8; F = 1.85; p = 0.300, respectively). However, significantly greater numbers of dusky damselfish were seen at the bottom of patch reefs (one-way block ANOVA on ranked data; df = 2,16; F = 3.35; p = 0.081, SNK MRT, alpha of 0.10). Yellowtail damselfish were seen in the fewest number of patch reef plots (9). Dusky damselfish were observed at fewer patch reef plots (17) than cocoa damselfish (22), even though both species occurred at similar average densities within the study area (Table 1).

Dusky damselfish and cocoa damselfish average densities were not significantly different from each other; but were significantly greater than yellowtail damselfish density (Fig. 3) across the study site (one-way block ANOVA on ranked data, df = 2,32; F = 6.76; p = 0.002, SNK MRT, alpha of 0.10). Mean respective densities (±SD) of dusky, cocoa, and yellowtail damselfishes within the patch reef study area were 0.33 (±1.739), 0.32 (±1.537), and 0.06 (±0.475) fish/m^2. While adults of the three dominant damselfish species occurred commonly throughout the patch reefs of Loggerhead Key, only cocoa damselfishes juveniles were consistently present in observation plots. No significant differences between adult and juvenile cocoa damselfish densities were found (t-test, t = 4.11; p = 0.246). Conversely, juvenile yellowtail damselfish were never present and dusky damselfish juveniles were seen on only three plots (Fig. 3).

DISCUSSION—Branching coral formations in the Dry Tortugas have been slow to recover from the catastrophic cold and disease events of the late 1970s and 80s. More than ten years later, most of the once extensive staghorn coral formations on the reef's outer margins are still little more than expanses of broken coral rubble. Although estimates of damselfish densities from DTNP are nonexistent, studies from the Florida Keys, approximately 175 km to the east of the Dry Tortugas, may provide useful comparative insights. Emery (1973) reported respective dusky, cocoa, and yellowtail damselfish densities on Alligator and Looe Key reefs of 0.33, 0.11, and 0.01 fish/m^2 from reef habitats similar to those we studied in the DTNP. Respective densities for these same fish in patch reef formations off Loggerhead Key in 2002 were 0.33, 0.32, and 0.06 fish/m^2 (Fig. 3). Although statistical comparisons between the two data sets could not be made (Emery's 1973 data did not include standard deviations), it is clear that cocoa and yellowtail damselfish densities were higher in DTNP than those reported by Emery (1973). The massive loss of branching coral habitat could account

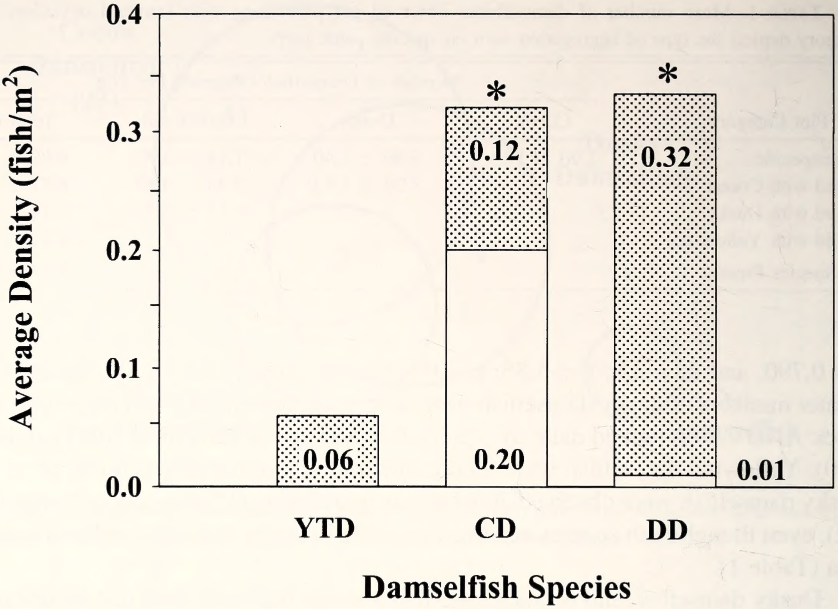

FIG. 3. Average patch reef densities of yellowtail damselfish (YTD), *Microspathodon chrysurus*, cocoa damselfish (CD), *Pomacentrus variabilis*, and dusky damselfish (DD), *Pomacentrus fuscus*, in Dry Tortugas National Park, Florida, USA, 2002. Numbers on bars represent adult (stippled bar), and juvenile (solid bar) densities in fish/m^2 for the respective species. Asterisks indicate total fish (adult +juvenile) densities that are not significantly different from one another.

for the increased densities we observed; however, without pre-impact data from DTNP, this hypothesis could not be tested. Nevertheless, the substantial alteration of reef habitat has almost certainly impacted damselfish populations in the Park.

It seems reasonable to assume that the higher damselfish densities on the surviving patch reefs in DTNP would increase selective pressure to effectively partition resources and space. Damselfish distribution at DTNP was distinctively patchy, showing a strong proclivity for monospecific aggregations. Over one-half of all plots observed (55%) contained a single damselfish species (Fig. 2), suggesting that interspecific competition for space and resources may have been reduced by horizontally partitioning patch reef environments. Partitioning between reefs was by no means absolute, however, and conspecific assemblages containing two damselfishes were found on 35% of plots with all three species coexisting on the remaining 10% of plots. Competitive interactions between species for space on patch reefs no doubt influences damselfish distributions (Emery, 1973), and may have played a key role in establishing distribution patterns within DTNP following the collapse of staghorn habitats. Cocoa damselfish were the most widely distributed of the three species, occurring on two-thirds (67%) of all patch reefs followed by dusky damselfish (52%) and yellowtail damselfish (27%). The relatively widespread distribution of cocoa damselfish in DTNP may be related to their exceptional ability to colonize marginal habitats. Fangue and co-workers (2001), for example, observed cocoa damselfish inhabiting rockpools on Loggerhead Key under hyperthermic and hypoxic conditions so harsh that they excluded nearly all other fish species. In addition, the presence of juvenile cocoa damselfish, on the patch reefs, indicates that this species may claim territories early in their life history, perhaps leaving fewer open habitats for dusky and yellowtail damselfishes that apparently recruit to the reef only as adults (Fig. 3).

While damselfishes at DTNP more or less partitioned patch reefs into single-species groups, vertical partitioning of reef habitat was less obvious. Emery (1973) found that dusky, cocoa, and yellowtail damselfishes on Alligator and Looe Key reefs showed a weak, but identifiable, vertical stratification, with each species preferring to inhabit a particular vertical dimension of patch reef. Similarly, we found that dusky damselfish preferred the bottom habitat of patch reefs to top or middle regions ($p = 0.081$). In contrast, we found no significant differences in cocoa or yellowtail damselfish density between top, middle, or bottom reef position. Regional differences in vertical preference between damselfish populations in the Florida Keys and those in DTNP cannot be ruled out; however, it is also possible that greater interspecific competition may override preference behaviors in these fishes when space becomes limiting. Bohnsack (1983) suggested that significant loss of fish species following cold spells might reduce immediate interspecific competition for some fishes; however, our density data suggest higher levels of interspecific competition, which may disrupt natural vertical partitioning by prompting damselfishes to exploit unfamiliar territories.

The classical view of coral reefs as steady state communities has recently been challenged by the argument that system diversity is actually maintained through periodic natural disruptions and catastrophic coral mortality (Sumich, 1999). Certainly cold weather events have played a major role in shaping the physical structure and diversity of the Florida reef tract, with cold fronts severe enough to affect coral mortality being recorded at approximately 10-year intervals since the 1930s (Storey 1937; Galloway 1941; Gunter and Hildebrand 1951; Roberts et al., 1982). Disease events may be a less regular, but equally important modifying factor as well. Territorial damselfishes have an obvious dependence on reef structural dynamics and it is not surprising that they have evolved strategies that allow them to exploit a variety of physical reef configurations. Indeed, dusky, cocoa, and yellowtail damselfishes seem well adapted to altering their distribution and habitat preference patterns to accommodate periodic massive changes in reef structure.

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LITERATURE CITED

AMERICAN FISHERIES SOCIETY. 1991. Common and Scientific Names of Fishes, American Fisheries Society Special Publication 20, 5th ed., Bethesda, MD.

ARONSON, R. B. AND W. F. PRECHT. 1997. Stasis, biological disturbance, and community structure of a Holocene coral reef. Paleobiology 23(3):326–346.

BOHNSACK, J. A. 1983. Resiliency of reef fish communities in the Florida Keys following a January 1977 hypothermal fish kill. Environ. Biol. Fish. 9(1):41–53.

DAVIS, G. E. 1982. A century of natural change in coral distribution at the Dry Tortugas: A comparison of reef maps from 1881 and 1976. Bull. Mar. Sci. 32(2):608–623.

EMERY, A. R. 1973. Comparative ecology and functional osetology of fourteen species of damselfish (Pisces: Pomacentridae) at Alligator Reef, Florida Keys. Bull. Mar. Sci. 23(3):649–770.

FANGUE, N. A., K. E. FLAHERTY, J. R. RUMMER, G. COLE, K. S. HANSEN, R. HINOTE, B. L. NOEL, H. WALLMAN, AND W. A. BENNETT. 2001. Temperature and hypoxia tolerance of selected fishes from a hyperthermal rockpool in the Dry Tortugas, with notes on diversity and behavior. Caribb. J. Sci. 37(1–2):81–87.

GALLOWAY, J. C. 1941. Lethal effects of the cold winter of 1939–40 on marine fishes at Key West, Florida. Copeia 1941(2):118–119.

GUNTER, G. AND H. H. HILDEBRAND. 1951. Destruction of fishes and other organisms on the south Texas coast by the cold wave of January 28-February 3, 1951. Ecology 32:731–736.

LIESKE, E. AND R. MYERS. 1999. Coral Reef Fishes, Princeton University Press, Princeton, NJ.

MCGEHEE, M. A. 1995. Juvenile settlement, survivorship and in situ growth rates of four species of Caribbean damselfishes in the genus *Stegastes*. Environ. Biol. Fish. 44:393–401.

PORTER, J. W., J. F. BATTEY, AND G. J. SMITH. 1982. Perturbation and change in coral reef communities. Proc. Natl. Acad. Sci. 79:1678–1681.

ROBERTS, H. H. AND L. J. ROUSE, JR. 1982. Cold-water stress in Florida Bay and northern Bahamas: A product of winter cold-air outbreaks. J. Sed. Pet. 52(1):0145–0155

ROBERTSON, D. R. 1984. Interspecific competition controls abundance and habitat use of territorial Caribbean damselfishes. Ecology 77(3):885–899.

STOREY, M. 1937. The relation between normal range and mortality of fishes due to cold at Sanibel Island Florida. Ecology 18:10–26

SWEATMAN, H. P. A. AND J. ST. JOHN. 1990. Effects of selective settlement and of aggression by residents on distribution of young recruits of two tropical damselfishes. Mar. Biol. 105:247–252.

SUMICH, J. L. 1999. An Introduction to the Biology of Marine Life, 7th ed. McGraw-Hill, Boston, MA.

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FISH AND WOOD STORK (*MYCTERIA AMERICANA*) POPULATION MONITORING IN TWO LARGE MOSQUITO IMPOUNDMENTS IN THE NORTHERN INDIAN RIVER LAGOON, FLORIDA: THE DYNAMICS OF ESTUARINE RECONNECTION

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Abstract: *Two large mosquito impoundments in the northern Indian River Lagoon, Florida were monitored before and after reconnection to the estuary with culverts and installation of pumps. Studies on hydrography, fish and wood stork (*Mycteria americana*) utilization were completed. After reconnection, the cells were managed differently, with management in one cell designed to enhance fish populations and attract wood storks. Following culvert and pump installation, salinities were significantly higher in both cells, and replication of estuary water levels was achieved during culvert "open" periods. Ten species of fish were collected prior to culvert reconnection and 12 after reconnection. There was no difference between total number or density of fishes before or after reconnection, and total number and density of fishes was greater in the modified RIM cell than in the RIM cell during one year only. Wood storks were not attracted by the winter-spring pump-up. This study demonstrates that even very large mosquito impoundments located far from tidal influences can be managed for multiple purposes with the installation of sufficient culverts and/or pumps, but the "target" species for management may not respond over short time scales.*

Key Words: waterbirds, Florida, estuary, saltmarsh, salinity, marsh restoration, wood stork

Wetlands are important reservoirs of biodiversity and offer economic, aesthetic and varied environmental benefits. Estuarine wetlands, in particular, may be highly productive and provide nursery, foraging and refuge habitats for many birds and commercially important fish and crustacean species (Weinstein, 1979; Dale and Hulsman, 1990; Rey et al., 1990a). Since many estuarine wetland areas have been or are now directly or indirectly affected by human activities, the management of these wetlands for optimum function and natural resource benefits is of increasing importance.

In east central Florida, the Indian River Lagoon (IRL) is a regionally and nationally important estuary and supports one of the most diverse biotas recorded from any estuarine area (Gilmore, 1985; Swain et al., 1995). Saltmarshes and mangrove swamps border many areas of the IRL, and much of this marsh acreage was impounded for mosquito control in the 1950–60s. Impoundment construction prevents oviposition by the floodwater saltmarsh mosquitos *Ochlerotatius taeniorhynchus* (Wiedemann) and *Oc. sollicitans* (Walker), the main pest mosquito species in coastal Florida. Impoundments were built by erecting a dike, excavated from an internal perimeter ditch, which encircled the marsh or connected with uplands, thus isolating the marsh from the adjacent estuary. The impoundments were then flooded with pumps using estuarine water, fresh-water from artesian wells or passive retention of rainwater. Flooding during the mosquito breeding season (May–October) provided an effective barrier against mosquito oviposition, as eggs must be laid on exposed, moist soils. Consequently, pesticide usage declined with this "source reduction" method (Provost, 1977; Carlson et al., 1991).

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Current addresses: 5 Running Springs Rd. Gorham ME 04038 (AB); Carroll and Associates, 1160 38th Ave., Vero Beach, FL 32960 USA (JDC).

However, in spite of the ecological benefits of reduced pesticide use, impoundment had negative effects on marsh ecology. Vegetation communities were drastically altered, or in many cases, completely eliminated, by over-flooding or hypersaline conditions resulting from excessive pumping followed by evaporation of saline water. In other cases, vegetation transitioned to freshwater communities by the lower salinities resulting from rainfall or artesian well flooding (Clements and Rogers, 1964; Gilmore et al., 1982; Harrington and Harrington, 1982). In the southern part of the IRL, the prolonged hydroperiods of early mosquito impoundment management resulted in nearly monospecific stands of the red mangrove (*Rhizophora mangle* L.), the mangrove species most tolerant of high water levels (Rey et al., 1990b).

The IRL estuary itself has likely been affected by impoundment construction, as 16,200 ha of impoundments were ultimately constructed, representing almost 90% of the marsh acreage remaining along the IRL (Rey and Kain, 1990). In particular, dike construction prevented the exchange of tidal water between the marsh and estuary, now recognized as an important component of estuarine function. This effect is most readily illustrated amongst fishes, where the number of fish species utilizing marshes decreased from 16 to 5 after impoundment construction (Harrington and Harrington, 1961). Many of the fishes denied impoundment access had commercial or sport fishery importance (e.g. tarpon, *Megalops atlanticus* Valenciennes; snook, *Centropomus undecimalis* Bloch; and mullet, *Mugil* spp.). The eliminated species had formerly utilized the marsh habitat during the fall and early winter months, when IRL water levels are high, and the marshes would have been flooded naturally (Gilmore et al., 1982; Harrington and Harrington, 1982).

Some of the deleterious effects of impoundment have been surmounted by the implementation of Rotational Impoundment Management (RIM). RIM entails the installation of culverts with water control structures through the dikes. The culverts are opened to allow estuarine water exchange during the non-mosquito breeding season (October-April), facilitating movement of nutrients and organisms between the marsh and estuary. The marsh could still be flooded for mosquito control during the summer months (May-October), and RIM appears to be a compromise between mosquito control and natural resource interests (Carlson and Carroll, 1985; Carlson et al., 1991).

Water birds appear to have benefitted from impoundment construction. Several studies (Trost, 1968; Breininger and Smith, 1990; Smith and Breininger, 1995; Schikorr and Swain, 1995) have shown that wading birds have adapted to the foraging and nesting opportunities provided by impoundments. Feeding in impoundments seems to be enhanced during low water conditions, when prey items are more concentrated, and the IRL and its mosquito impoundments are viewed as a regionally important resource for wading birds (Swain and Shenker, 1993; Sewell et al., 1995). Since water levels can be easily manipulated in some RIM-managed impoundments, "drawdowns" can attract wading birds and provide improved feeding opportunities, particularly during the breeding season (Rosier, 1993; Swain and Shenker, 1993). Both shorebirds and waterfowl also use impounded wetlands along the IRL. Ducks utilize impoundments managed by flooding during the winter months, and shore birds feed on the exposed mud flats of impoundments that are drawn down during spring migration (Breininger and Smith, 1990).

The woodstork (*Mycteria americana* L.) is one species of wading bird that may benefit from feeding opportunities provided by managed wetlands and changing water levels. Wood storks have been federally listed in four southeastern USA states since 1984. Only 5,000–6,000 breeding pairs were thought to remain in the mid-1980s (Ogden et al., 1987), but the population may now have increased to 14,000–20,000 individuals (Bancroft et al., 1992). Wood stork populations, which used to be centered in south Florida Everglades' habitats, appear to have shifted northward, due to declining habitat conditions and nesting failure in the Everglades (Ogden et al., 1987), and the number of birds in central Florida has doubled since 1960 (Ogden, 1991). Food availability appears to be highly correlated with nesting success in wood storks (Coulter and Bryan, 1995), so considerable research has been done on the feeding preferences of this animal (Kushlan et al., 1975; Ogden et al., 1987; Depkin et al., 1992; Coulter and Bryan, 1993).

Wood storks in central Florida begin nesting in mid-March to late-April and will fly for considerable distances (90 km) to forage for nestlings (Bancroft et al., 1992). The ready availability of a food supply to a breeding colony may be of paramount importance. Wood storks typically have several nesting colonies along the IRL, mostly in the southern portion. In the northern IRL, the impoundments around Kennedy Space Center historically contained about 10% of the Florida nesting population (Clark and Lee, 1982; Swain and Shenker, 1993). However, a series of severe freezes in the late 1980s destroyed the larger mangroves that were utilized for nesting, and many of the colonies in this region were abandoned (Smith and Breininger, 1995).

Since most mosquito impoundments are now actively managed, they provide opportunities for wildlife enhancement. Many studies have been completed on the effects of reconnecting mosquito impoundments, specifically on the effects of RIM management (reviewed in Brockmeyer et al., 1997). However, most of these studies have taken place in the mangrove-dominated southern portion of the IRL, where impoundments have more tidal influence, and most have focused on specific aspects of impoundment "recovery" (i.e., fishes) in smaller impoundments. Few studies have addressed the effects of reconnection of large impoundments in the grassy marshes of the northern IRL, and fewer still have examined the responses of both fish and birds to impoundment manipulation. The following multi-year study describes the effects of impoundment reconnection and RIM management in two large impoundments in the microtidal, northern IRL, Florida. The study relates the overall effects of impoundment reconnection on fishes, salinity and hydrography. In addition, the effects of a novel water management strategy, flooding the impoundment during winter-spring, on fish density and wood stork utilization was investigated. Specifically, we wished to determine the following: (1) the effects of culvert installation on hydrography and salinity; (2) changes in fish species diversity, population density and habitat use patterns following reconnection; and (3) whether fish population density could be enhanced by a winter-spring pump-up, thus attracting wood storks during their breeding season.

MATERIALS AND METHODS—A multi-year study was completed in two cells of the Sykes Creek Impoundments on Merritt Island, Florida, USA (28° 25′ N, 80° 40′ W) (Fig. 1). The two impoundments (north and south cells) consist of 308 ha (north) and 534 ha (south). The impoundments are surrounded by urban and suburban development typical for this part of Florida. The cells were formerly part of a saltmarsh at the headwaters of Sykes Creek, a tributary of the Banana River (part of the IRL system), but were bisected by a highway and canal system. The marshes had been isolated from the adjacent Banana River since the dikes were erected in 1956–58 and were kept flooded year-round by intermittent pumping with two small pumps, as well as retention of rainfall (Rey and Kain, 1990). No estuarine connection was established, other than one small culvert in each cell which was never opened, and the impoundments were managed for mosquito control only. The impoundments were vegetated primarily with the saltgrasses (*Distichlis* and *Paspalum*) with other limited coverage by black needlerush (*Juncus*), cattail (*Typha*), leatherfern (*Acrostichum*) and some mixed mangroves (*Rhizophora*, *Avicennia*, *Laguncularia*). Topography in the two cells was similar, with large areas of marsh flat interspersed with numerous large and small ponds and relict tidal creek channels. About 20% of each cell consisted of ponds and creeks (Rey and Kain, 1990, Fig. 1.)

In the early 1980s, as part of a mitigation plan developed by the U.S. Army Corps of Engineers to compensate for nearby estuarine wetland impacts, these two marshes were proposed for restoration. The mitigation plan has been described in detail elsewhere (Banner and Moulding, 1987; Taylor, 1989), but essentially called for the installation of multiple culverts and a single large pump (pumping capacity: north cell-45,360 l/min; south cell: 94,500 l/min) in each cell. One cell (north) would be operated under standard RIM protocol (pump-up in the summer and open culverts in fall-winter-spring). The south cell would be operated under a modified RIM plan, in which the culverts would also be closed in early January and the impoundment flooded by pumping. The water level would then be slowly dropped during Jan–April, with the assumption that the declining water levels would attract breeding wood storks to feed upon the concentrated fish populations. A detailed monitoring program was designed with the mitigation plan, and sampling stations were established for fish sampling, salinity and water level recording, and wood stork counts. The design of the monitoring program was as follows:

Timeline

The pre-connection sampling period was conducted from October 1986 to November 1987, although water level recordings were continued until March, 1988. There was a subsequent delay in completion of the mitigation plan and installation of the culverts (Taylor, 1989), and culverts were not installed in the north cell until 1989 (14 culverts; 91 cm dia) and in the south cell in 1992 (32 culverts; 91 cm dia). The post-connection monitoring in both cells began in December 1992 and continued until December 1994.

Sample Stations

Within each cell, 9 stations were designated within three broad categories: permanent ponds (ponds which never dried and had historically been linked to the estuary by a channel), ephemeral ponds

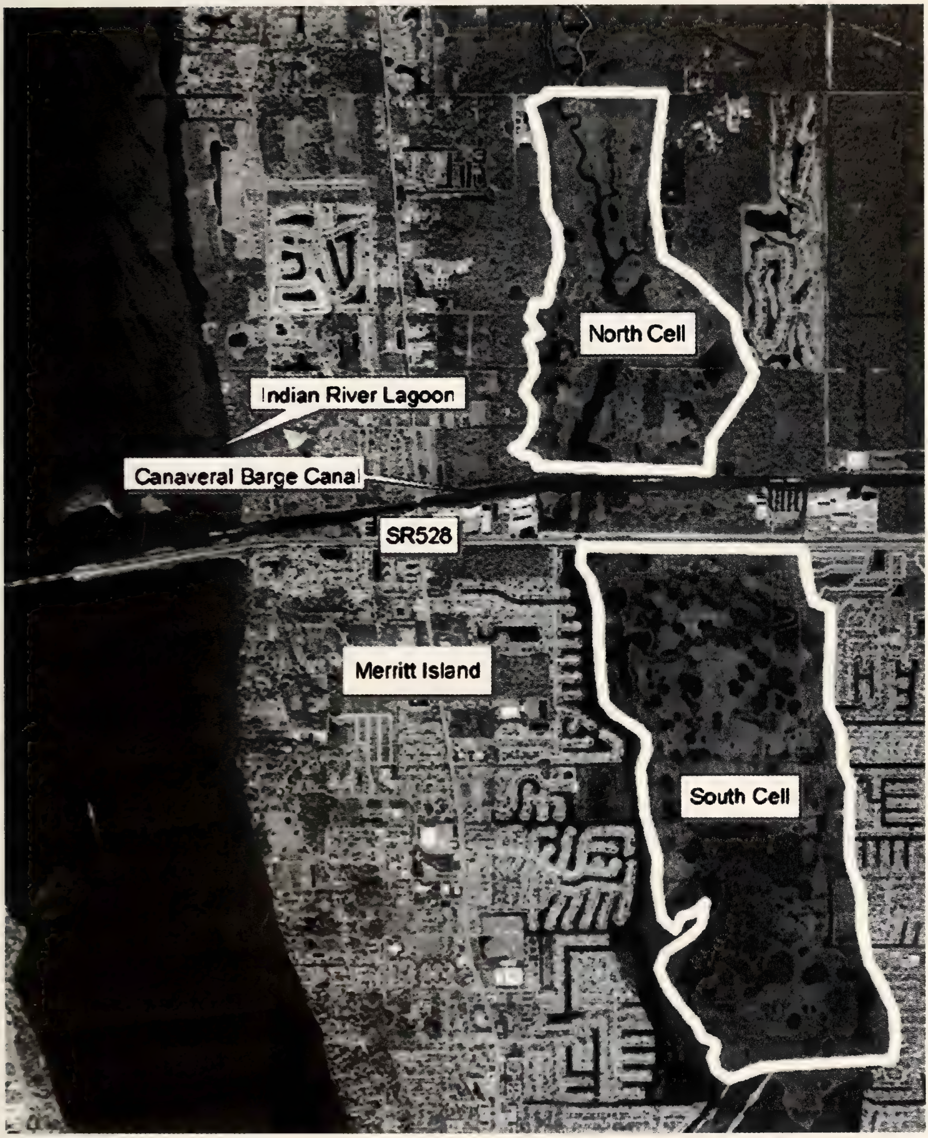

FIG. 1. The Sykes Creek impoundments, north and south cells.

(seasonally flooded ponds, isolated by stands of emergent wetland vegetation), and high marsh (vegetated habitats which were intermittently dry). In the north cell, three ephemeral, four permanent and two high marsh stations were established; within the south cell, two ephemeral, four permanent and three high marsh stations were established. In addition, a single station in the open estuary adjacent to the south cell was established for estuary water level monitoring. Stations were visited monthly during the two sampling periods (pre- and post-connection), using either a canoe or, on rare occasions, an airboat traveling a defined transect. Some high marsh stations could not be reached during low water conditions, and were assumed to be dry and thus not sampled.

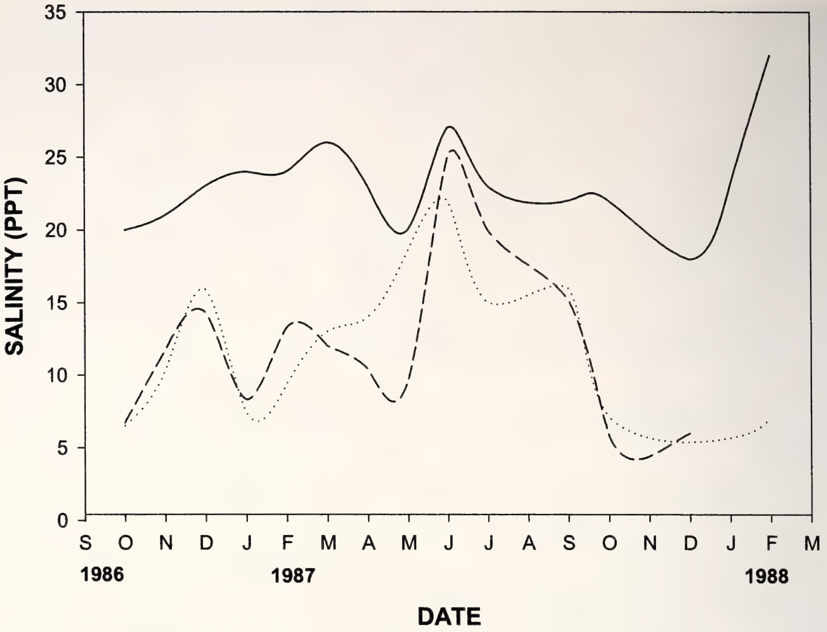

FIG. 2. Salinity (ppt) in both cells of the Sykes Creek impoundments and the estuary. Pre-connection (1986–88). North Cell = ..., South Cell = – – –, Estuary = ———.

Salinity/water level—Salinity was recorded at each station during each sampling event with an optical refractometer. Continuous water level recordings were made with Stevens Water Level Recorders (Model F) at both the estuary station and within each cell. Water level recordings were transcribed and converted to meters NGVD.

Fishes—Fishes were sampled at each station with either a 106 cm dia., 7 mm mesh cast net or a 0.33 m^2 aluminum throw trap. The cast net was used at all permanent pond sites and at ephemeral pond sites where water depth was sufficient (> 8 cm). Three random throws of the net were completed at each station. Fishes were preserved in formalin in the field for later identification, enumeration and measurement. Throw traps were used at all high marsh stations, where thick vegetation prevented use of the cast net. The specific throw traps used are efficient in shallow marine settings, and have been used elsewhere to demonstrate positive correlations between foraging attempts by wading birds and fish density (Miranda and Collazo, 1997). The throw trap was also thrown randomly three times. The contents of the trap were removed by repeated sweeps with a frame net. The trap was assumed to be empty when three successive sweeps were devoid of fishes. Fishes from this trap were handled as above.

Birds—The presence/absence of wading birds (Order Ciconiiformes) in a 250 m^2 area within each station was noted during each sampling event, and species were identified. On the rare occasion when an airboat was used, the boat was halted at a distance to avoid startling the birds. In addition, the total number of wood storks was counted throughout the transect in each cell.

Data analyses—Salinities for each cell were compared pre- and post-connection with non-parametric T-tests (Mann-Whitney U-test) and water levels in one cell during open culvert periods were compared to the open estuary with a Spearman correlation. Fish numbers and densities were compared

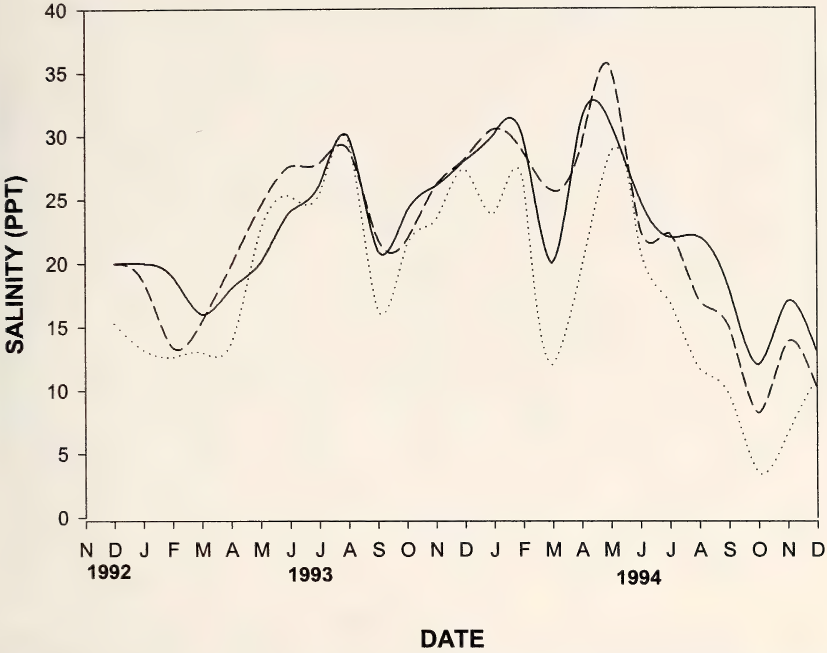

FIG. 3. Salinity (ppt) in both cells of the Sykes Creek impoundments and the estuary. Post-connection (1992–94). North Cell = ..., South Cell = – – –, Estuary = ———.

between cells and years by ANOVA, as were numbers and densities within the different habitat types. Mann-Whitney tests and parametric t-tests were used to compare the effects on fishes of the winter pump-up and between cells over all years. Numbers of wood storks were correlated with water levels in each cell and parametric t-tests compared number of storks between cells during pump-up and over all years. All significance levels were established at $p \leq 0.05$.

RESULTS—*Salinity/water level*—During the pre-connection monitoring (1986–88), salinities at all impoundment stations ranged from a minimum of 1 ppt to 24 ppt (Fig. 2) and during post-connection (1993–1994) from 2–40 ppt (Fig. 3). When salinities for all stations within each cell were compared during the pre-connection to post-connection periods with Mann-Whitney U-tests, both cells had significantly higher salinities following reconnection (north cell: $T = 9128.5$; $p < 0.001$: south cell: $T = 2532.0$; $p < 0.001$). Rainfall, as measured at Titusville, Florida (25 km from the study site), was very low in 1986–87 (deficit of 41.4 cm and 16.0 cm, respectively) and 1993 (deficit of 35.3 cm), but extremely high in 1994 (excess of 61.6 cm) (NOAA 1986, 1987, 1993, 1994).

Water level—There was considerable disparity between water level in the IRL and in both cells of the impoundment, especially during the fall of 1987 when heavy rains raised the impoundment water levels well above that of the IRL (Fig. 4). Also, since there was no culvert connection between either cell and the estuary, there was

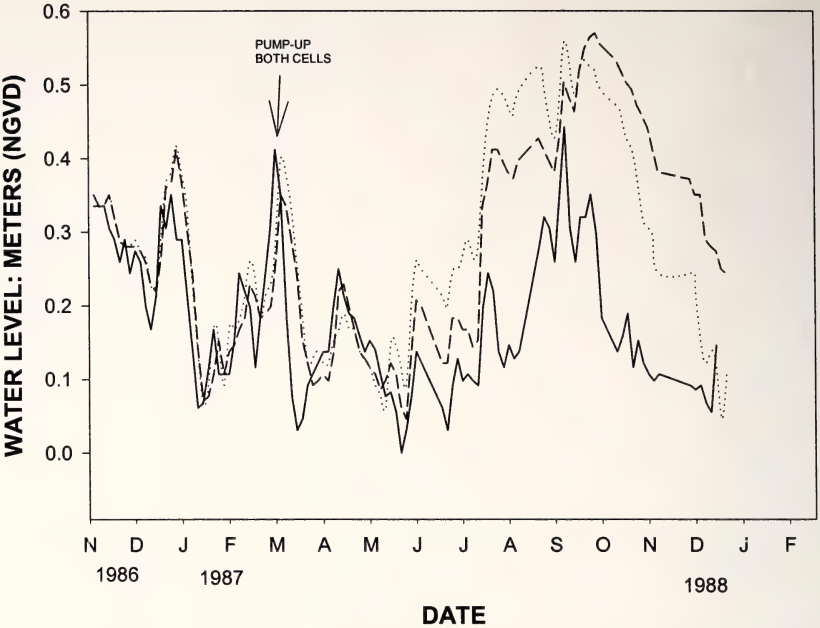

FIG. 4. Water level (meters NGVD) in both cells of the Sykes Creek impoundments and the estuary. Pre-connection (1986–88). North Cell = ..., South Cell = – – –, Estuary = ———.

little replication of estuary water levels within the impoundments and no way for excess water resulting from heavy rainfall to exit the cells.

Following reconnection, water level peaked and subsequently declined in the south cell during winter-spring pump-up (Jan–March) for each year, and during the pump-up for mosquito control in both cells during the summer of both years (Fig. 5). Both cells fluctuated with the estuary water level during the fall-winter-early spring months (open culverts). For example, in the north cell, culverts were opened in October 1993 and closed in June 1994. During the open period, this cell fluctuated almost uniformly with the estuary (Spearman r = 0.925; p = 0.00). A series of storm events brought IRL water level to a very high maximum in the fall of 1994.

Fish sampling—During pre-connection (1986–87), stations were sampled for fishes 148 times, while in post-connection 199 and 158 samples were taken during 1993 and 1994, respectively. The number of samples collected was not significantly different between the three periods (ANOVA; F = 1.1; P = 0.34). During the entire study, a total of 7,979 individual fish from 14 species was collected inside the impoundments (Table 1). By far, the three most abundant species inside the two impoundments were *Cyprinodon variegatus* Lacepéde (n = 3,075), *Poecilia latipinna* (Lesueur) (n = 2,668), and *Gambusia holbrooki* (Baird and Girard) (n = 1,849). These species comprised 95% of the total catch by number. Ten species were collected pre-connection, and 12 post-connection. Two species of freshwater affinity

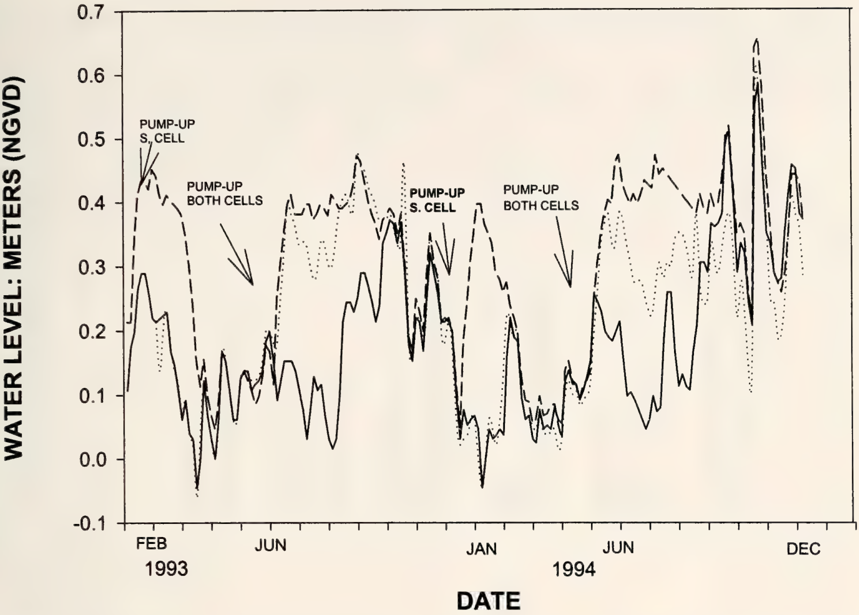

FIG. 5. Water level (meters NGVD) in both cells of the Sykes Creek impoundments and the estuary. Post-connection (1992–94). North Cell = ..., South Cell = – – –, Estuary = ———.

Jordanella floridae (Goode and Bean) and *Heterandria formosa* Agassiz, were not taken following reconnection, and four species of estuarine affinity were added in the post-connection period: *Gobionellus shufeldti* (Jordan and Eigenmann), *Tilapia melanotheron* (Rüppell), *Archirus lineatus* (L.) and *Mugil curema* (Valenciennes). Several other estuarine species were incidently noted inside the impoundments during the post-reconnection period (Table 1), indicating further recruitment via culverts from the estuary.

When collapsed across marsh cells, all habitats and all species, there was no significant difference (ANOVA) between years (1986–87, 1993, 1994) for total number ($F = 1.90$; $P = 0.16$) or density ($F = 0.04$; $P = 0.96$) of fishes. The density of fishes for each marsh habitat (permanent pond, ephemeral pond, high marsh) across both cells for all years is shown in Fig. 6. When data were collapsed across cells, an examination of fish density by habitat type for each period (1986–87, 1993, 1994) indicated no significant differences between habitat types for 1993 and 1994 (ANOVA: $F = 2.25$ and 2.90; $P = 0.13$ and $P = 0.08$). However, for 1986–88, high marsh stations had higher fish densities ($\bar{x} = 24.5$ fish/m^2) than ephemeral ($\bar{x} = 8.1$ fish/m^2) or permanent ponds ($\bar{x} = 4.5$ fish/m^2) (ANOVA: $F = 3.99$; $P = 0.04$). There was no apparent effect on fish density following impoundment reconnection in 1989 (north cell) and in 1992 (south cell). When the north and south cells were compared by each habitat type for the three sampling periods, there were no significant differences in fish density (ANOVA: $F = 0.25$–2.62; $P = 0.15$–0.80).

Of particular interest were the effects of the winter pump-up in the south cell on average fish density collapsed across habitat type. When the north and south cells

TABLE 1. Fish species collected in the Sykes Creek Impoundments and Indian River Lagoon during two sampling periods: 1986–88 (pre-connection) and 1993–1994 (post-connection). *** Denotes presence of species.

Fish species	1986–88	1993–94	Incidental observation in impoundments (post-connection)	Estuary
Gambusia holbrooki	***	***		
Poecilia latipinna	***	***		***
Cyprinodon variegatus	***	***		***
Fundulus confluentus	***	***		***
Fundulus grandis	***	***		
Jordanella floridae	***			
Heterandria formosa	***			
Lucania parva	***	***		***
Menidia spp.	***	***		***
Mugil cephalus	***	***		***
Mugil curema		***		***
Tilapia melanotheron		***		***
Archirus lineatus		***		***
Oligoplites saurus			***	***
Gobionellus schufeldti		***		
Elops saurus			***	
Cynoscion nebulosus			***	
Megalops atlanticus			***	
Centropomous undecimalus			***	
Brevoortia sp.			***	
Lepisosteus sp.			***	

were compared for this period during both years (Jan–April, 1993 and 1994), there was no difference between the north (non-pumped) and south (pumped) cells in either total number of fish collected during 1993 (Mann-Whitney: T = 951.0; p = 0.09) or fish density (Mann-Whitney: T = 1019.0; p = 0.20). However, in 1994 both number of fish and density were significantly higher in the south cell (total number: Mann-Whitney: T = 302.5; p = 0.004: density: Mann-Whitney: T = 505.0; p = 0.002) (Fig. 7).

When averaged across habitat type, the total number of fish collected during pre-connection was not significantly different from either of the post-connection years within the north cell (1993 t-test: t = –1.13; p = 0.27: 1994: t = –0.16; p = 0.88). The same was true for the south cell during 1993 (t = –1.19; p = 0.25). In 1994, the number of fish was higher in the south cell than during pre-connection (t = –3.03; p = 0.009).

Overall, fish densities within the north cell did not vary between the pre- and post-connection samples (pre- vs. 1993: Mann-Whitney: T = 105.0; p = 0.97: pre- vs. 1994: T = 111.0; p = 0.68), and the same was true for the south cell (pre- vs. 1993: t-test: t = 0.62; p = 054: pre- vs. 1994: Mann-Whitney: T = 58.0; p = 0.32).

Bird counts—A species list of wading birds is shown in Table 2, and 12 species were recorded.

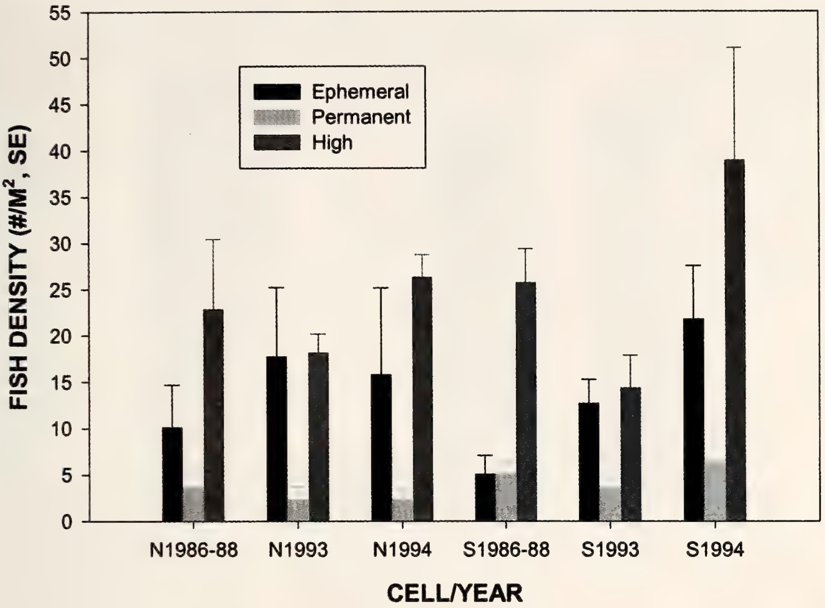

FIG. 6. Mean fish density {#m^2(se)} in three different marsh types of both cells of the Sykes Creek impoundments. Pre- and post-connection. N = North, S = South.

Wood storks- During the pre-connection monitoring period (1986–88), 45 wood storks were observed in the north cell ($\bar{x} = 3$ birds/month; sd = 7.71) and 59 in the south cell ($\bar{x} = 3.69$; sd = 6.01). In the post-connection monitoring, 163 birds were counted in the north cell ($\bar{x} = 6.52$; sd = 9.5) and 185 in the south cell ($\bar{x} = 7.4$; sd = 11.18). There was no correlation between water level and wood stork count for the north cell (Spearman r = 0.26; p = 0.23) (Fig. 8), while in the south cell there was a marginal positive correlation between water level and wood stork count (Spearman r = 0.46; p = 0.02) (Fig. 9). During the post-connection monitoring period, there was no difference in the number of wood storks for the entire period between the north and south cells (t-test: t = 2.01; p = 0.76). In particular, during the two winter pump-up periods in the south cell (January–April, 1993 and 1994), there was no difference in wood stork numbers between the two cells (t-tests; t = 2.77; p = 0.65 and t = 2.78; p = 0.20, respectively).

DISCUSSION—Prior to reconnection, there was considerable disparity between salinities within both cells and the estuary. However, both cells showed increases in ambient salinity, closely tracking estuarine salinity, following reconnection by culverts, in spite of excessive precipitation in 1994 (Fig. 3). This trend may be attributed to the presence of the multiple culverts and to increased pumping capability of the larger pumps. Since this portion of the IRL is microtidal (Smith, 1987), the dominant period for movement of water between RIM-managed cells and the estuary occurs only during the fall-winter-spring period of open culverts. This "open" period

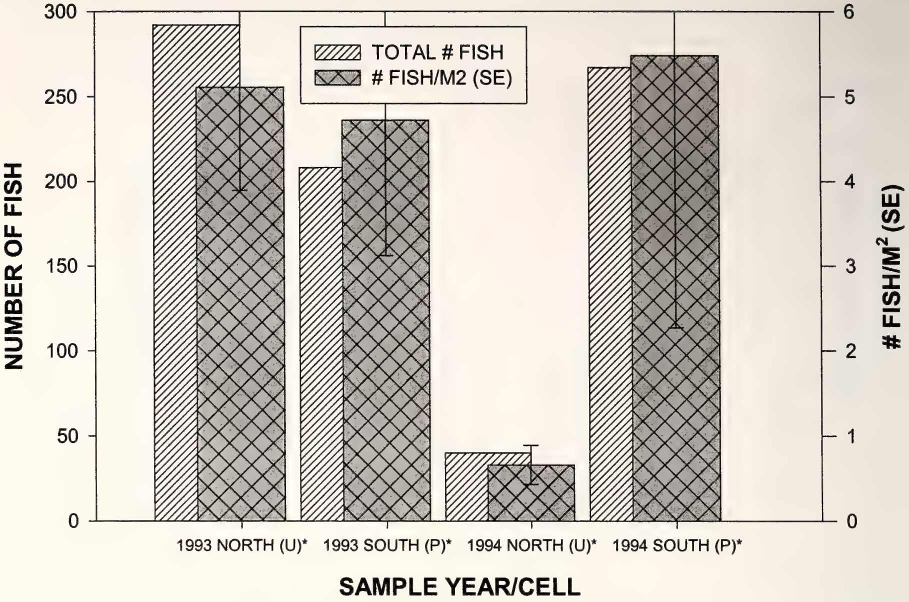

FIG. 7. Fish collections in the Sykes Creek impoundments during winter/spring pump-up (Jan–April 1993 and 1994). Total number and mean density {#m^2(se)} in north (unpumped) and south (pumped) cells. *U = Unpumped, *P = Pumped.

has resulted in increases in salinity in other impoundments along the IRL following installation of culverts (Rey et al., 1990c; Brockmeyer et al., 1997). The number of culverts was apparently sufficient to achieve excellent replication of water levels between the two cells and the estuary during open periods (Figs. 4 and 5).

Increasing salinities were probably responsible for the elimination of the two species of freshwater fishes not found in the post-connection studies (*J. floridae* and *H. formosa*). Another freshwater form which apparently disappeared from the south cell following reconnection was the crayfish (*Procambarus* sp.). Abundant burrows were observed and the animal collected throughout the marsh prior to reconnection (D.S.T, personal observation), but no burrows were observed nor were crayfish collected in the post-connection monitoring.

Other impoundment studies examining changes in fish fauna following culvert installation have often shown dramatic increases in fish species diversity (Rey et al., 1990c, Taylor et al., 1998; Poulakis et al., 2002). However, collections in the current study indicated the addition of only four new estuarine species, although six other estuarine species were incidently observed (Table 1). Fish collection techniques, while apparently adequate for ascertaining resident fish populations, may not have been geared toward the collection of transient species, which, upon entering impoundments, reside primarily in perimeter ditches and deeper, open areas (Taylor et al., 1998; Faunce and Paperno, 1999; Poulakis et al., 2002).

The fish data indicate that the cells were similar in fish numbers and that there was no difference between years, even pre- and post-reconnection. The winter

TABLE 2. Wading birds associated with the Sykes Creek Impoundments: 1986–88 and 1993–94.

Common Name	Species	Relative Abundance
Snowy Egret	*Egretta thula*	***
Little Blue Heron	*Egretta caerulea*	**
Tricolored Heron	*Egretta tricolor*	**
Reddish Egret	*Egretta rufescens*	*
Great Egret	*Casmerodius albus*	***
Great Blue Heron	*Ardea herodias*	*
White Ibis	*Eudocimus albus*	**
Glossy Ibis	*Plegadis falcinellus*	***
Least Bittern	*Ixobrychus exilis*	*
Cattle Egret	*Bubulcus ibis*	*
Green-backed Heron	*Butorides striatus*	*

*** Abundant.
** Common.
* Rare.

pump-up period in the south cell apparently enhanced fish number and density over the un-pumped state in the north cell only during the second year (1994). Fish density also did not differ greatly between habitat types, with the exception of a marginally higher density in high marsh stations during the pre-connection period. Within the two cells, there was also little variability in fish number or density over time. Only the south cell showed an increase in fish number during the second year of the post-connection study as compared to the pre-connection period, and this may be attributed to the winter pump-up period.

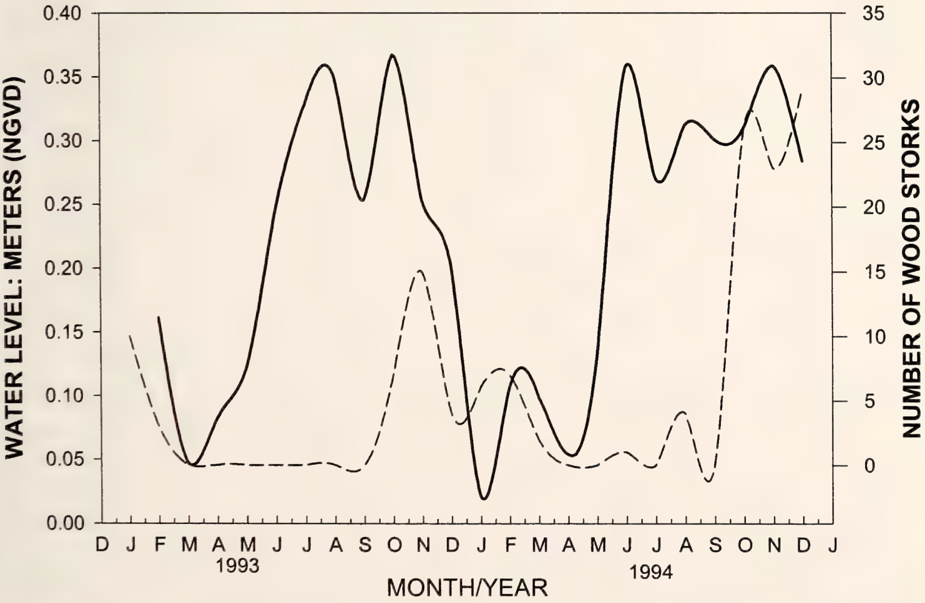

FIG. 8. Number of wood storks (*Mycteria americana*) and water level (meters NGVD) in the north cell of the Sykes Creek impoundments, 1993–94. ——— = Water level, – – – = # Wood storks.

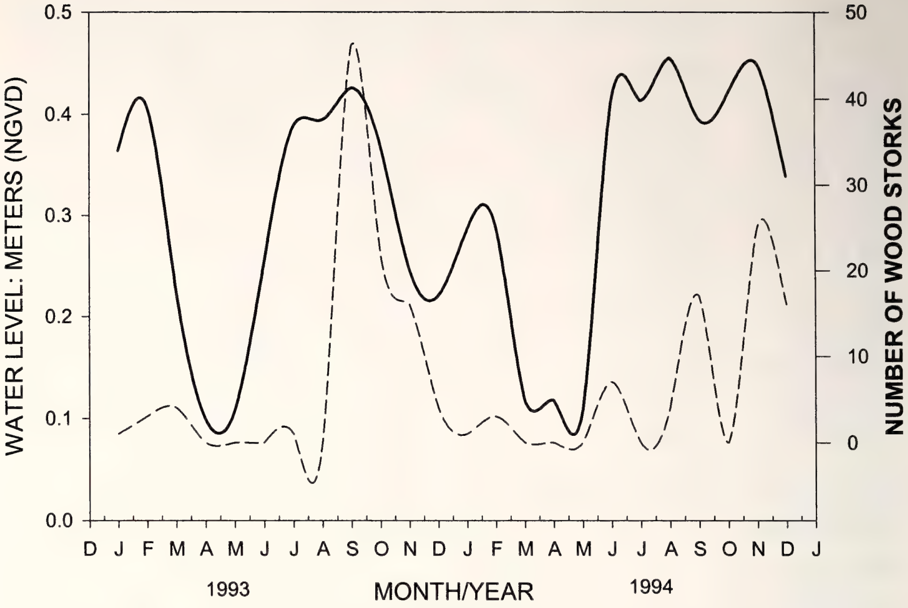

FIG. 9. Number of wood storks (*Mycteria americana*) and water level (meters NGVD) in the south cell of the Sykes Creek impoundments, 1993–94. ——— = Water level, – – – = # Wood storks.

The main goal of the novel water management regime in the south cell, attraction of wood storks during their nesting season, was apparently not successful, in spite of some evidence of enhanced fish production here. Wood stork counts were highly variable (Figs. 8 and 9). Further, the slight positive correlation between water level and stork numbers in the south cell was the opposite of that expected: stork numbers were hypothesized to increase with falling water level. The overall lack of difference in counts between the two cells during the winter pump-up is disappointing. This two-year study of wood stork response to habitat manipulation may have been too short in duration. O'Bryan and Carlson (1991) found that three years were required to attract wood storks to known feeding areas in another part of the IRL. Prey size and density could also have been factors. In the current study, 33% of fishes collected during the pre-connection period were >25 mm in length. In the 2-year post-connection study, 37% were >25 mm in length. Coulter and Bryan (1993) found that wood storks took only prey (fish, amphibians and crustaceans) greater than 25 mm mean length in a wetland in central Georgia. However, this mean prey length was almost twice that observed in Florida studies (Depkin et al., 1992). The median prey density in the Georgia study was 2.67 items/m^2, and while median prey densities were not calculated in the current study, mean densities certainly exceeded this value except for the north cell in 1994 (Fig. 7). Prey density, however, did not approach that observed in Everglades foraging sites (mean density: 141 items/m^2) (Kushlan et al., 1975; Ogden et al., 1976). In the Everglades, *Gambusia* comprised 60% of the biomass in pools, but were only 5% of the diet component of wood storks (Ogden et al., 1976). *Gambusia* were the third most abundant fish in the current study, but prey selectivity studies have not been done with IRL wood storks, and it is possible that the dominance of *G. holbrooki*, *P. latipinna* and *C. variegatus* in impoundment fish fauna has enhanced their selectivity by wood storks (Swain and Shenker, 1993). Low water conditions in the IRL and associated marshes are well-correlated with higher fish densities (Swain and Shenker, 1993; Taylor et al., 1998; Poulakis et al., 2002), but many questions remain about the effects of impoundment drawdowns on bird feeding strategies.

Whereas the main goal of this study, enhancement of feeding opportunities for wood storks, was apparently not successful, this project demonstrates that even very large mosquito impoundments located far from significant tidal influence can be managed for multiple purposes. However, the responses of organisms "targeted" for management may not occur over short time scales. Further efforts should be made to fine-tune management of impoundments for diverse purposes where the infrastructure (i.e., pumps, culverts) exists.

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LITERATURE CITED

Bancroft, G. T., W. Hoffman, R. J. Sawicki, and J. C. Ogden. 1992. The importance of water conservation area in the Everglades to the endangered wood stork (*Mycteria americana*). Cons. Biol. 6:392–398.

Banner, A. and J. Moulding. 1987. Mitigation management of an impounded brackish water marsh. Pp. 37–47. *In*: Webb, F. J., Jr. (ed.), Proceedings of the Fourteenth Annual Conference on Wetlands Restoration and Creation. Hillsborough Community College, Plant City, FL.

Breininger, D. R. and R. B. Smith. 1990. Waterbird use of coastal impoundments and management implications in east-central Florida. Wetlands 10:223–241.

Brockmeyer, R. E., Jr., J. R. Rey, R. W. Virnstein, R. G. Gilmore, and L. Earnest. 1997. Rehabilitation of impounded estuarine wetlands by hydrologic reconnection to the Indian River Lagoon, Florida (USA). Wetlands Ecol. and Mngmt. 4:93–109.

Carlson, D. B. and J. D. Carroll, Jr., 1985. Developing and implementing impoundment management methods benefitting mosquito control, fish and wildlife: a two year progress report about the Technical Subcommittee on Mosquito Impoundments. J. FL. Anti-Mosq. Assoc. 56:24–32.

———, P. D. O'Bryan, and J. R. Rey. 1991. A review of current salt marsh management issues in Florida. J. Am. Mosq. Cont. Assoc. 7:83–88.

Clark, E. S. and R. C. Lee. 1982. History and status of wood stork nesting on Merritt Island National Wildlife Refuge. Unpub. final rpt: Merritt Island National Wildlife Refuge, United States Fish and Wildlife Service, Titusville, Florida.

Clements, B. W. and A. J. Rogers. 1964. Studies of impounding for control of salt-marsh mosquitoes in Florida, 1958–63. Mosq. News 24:265–276.

Coulter, M. C. and A. L. Bryan, Jr., 1993. Foraging ecology of wood storks (*Mycteria americana*) in east-central Georgia 1. Characteristics of foraging sites. 1993. Colon. Waterbirds 16:59–70.

——— and A. L. Bryan, Jr., 1995. Factors affecting the reproductive success of wood storks (*Mycteria americana*) in east-central Georgia. Auk 112:237–243.

Dale, P. E. R. and K. Hulsman. 1990. A critical review of saltmarsh management methods for mosquito control. Rev. Aquatic Sci. 3:281–311.

DEPKIN, F. C., M. C. COULTER, AND A. L. BRYAN, JR., 1992. Food of nestling wood storks in east-central Georgia. Colon. Waterbirds 15:219–225.

FAUNCE, C. H. AND R. PAPERNO. 1999. *Tilapia*-dominated fish assemblages within an impounded mangrove ecosystem in east-central Florida. Wetlands 19:126–138.

GILMORE, R. G., JR., 1985. The productive web of life in the estuary. Pp. 52–72. *In*: Barile, D. D. (ed.). The Indian River Lagoon: Proc. of the Indian River Resources Symposium. Sea Grant Project No. I.R.84-28. Marine Resources Council of East Central Florida, Florida Institute of Technology, Melbourne, FL.

———, D. W. COOKE, AND C. J. DONOHOE. 1982. A comparison of the fish populations and habitat in open and closed salt marsh impoundments in east-central Florida. Northeast Gulf Sci. 5:25–37.

HARRINGTON, R. W., JR., AND E. S. HARRRINGTON. 1961. Food selection among fishes invading a high subtropical salt marsh from the onset of flooding through the progress of a mosquito brood. Ecology 42:642–666.

——— AND E. S. HARRINGTON. 1982. Effects on fishes and their forage organisms of impounding a Florida salt marsh to prevent breeding by salt marsh mosquitoes. Bull. Mar. Sci. 32:523–531.

KUSHLAN, J. A., J. C. OGDEN, AND J. L. TILMANT. 1975. Relation of water level and fish availability to wood stork reproduction in the southern Everglades, Florida. U. S. Geological Survey Open File Report 75-434, Tallahassee, FL.

MIRANDA, L. AND J. A. COLLAZO. 1997. A modified throw-trap to sample prey for wading bird studies. J. Field Ornithol. 68:602–606.

NOAA, 1986, 1987, 1993, 1994. National Oceanic and Atmospheric Administration, Environmental Data and Information Service, National Climatic Center, Asheville, North Carolina, USA.

O'BRYAN, P. D. AND D. B. CARLSON. 1991. Aerial bird surveys at Bird's (IRC #2) and Pete's (IRC #3) impoundments. Final report to U.S.F.W.S. Indian River Mosquito Control District, Vero Beach, FL.

OGDEN, J. C. 1991. Nesting by woodstorks in natural, altered and artificial wetlands in central and northern Florida. Colon. Waterbirds 14:39–45.

———, J. A. KUSHLAN, AND J. T. TILMANT. 1976. Prey selectivity by the wood stork. Condor 78:324–330.

———, D. A. MCCRIMMON, JR., G. T. BANCROFT, AND B. W. PATTY. 1987. Breeding populations of the wood stork in the southeastern United States. Condor 89:752–759.

POULAKIS, G. R., J. M. SHENKER, AND D. S. TAYLOR. 2002. Habitat use by fishes after tidal reconnection of an impounded estuarine wetland in the Indian River Lagoon, Florida (USA). Wetlands Ecol. Mngmt. 10:51–69.

PROVOST, M. W. 1977. Source reduction in salt-marsh mosquito control: past and future. Mosq. News 37:689–698.

REY, J. R. AND T. KAIN. 1990. A Guide to the Saltmarsh Impoundments of Florida. University of Florida, Florida Medical Entomology Laboratory, Vero Beach, FL.

———, M. S. PETERSON, T. R. KAIN, F. E. VOSE, AND R. A. CROSSMAN. 1990a. Fish populations and physical conditions in ditched and impounded marshes in east-central Florida. Northeast Gulf Sci. 11:163–170.

———, R. A. CROSSMAN, AND T. R. KAIN. 1990b. Vegetation dynamics in impounded marshes along the Indian River Lagoon, Florida, USA. Environ. Manag. 14:397–409.

———, J. SHAFFER, AND D. TREMAIN. 1990c. Effects of re-establishing tidal connections in two impounded subtropical marshes on fishes and physical conditions. Wetlands 10:27–45.

ROSIER, J. C. 1993. Wading bird feeding responses to manipulated water levels in Florida salt marsh impoundments. M.S. Thesis, Florida Institute of Technology, Melbourne, FL.

SCHIKORR, K. E. AND H. M. SWAIN. 1995. Wading birds-barometer of management strategies in the Indian River Lagoon. Bull. Mar. Sci. 57:215–229.

SEWELL, C. W., N. D. JOINER, M. S. ROBSON AND M. J. WEISE. 1995. Wading bird colony protection in the Indian River Lagoon. Bull. Mar. Sci. 57:237–241.

SMITH, N. P. 1987. An introduction to the tides of Florida's Indian River Lagoon, I. Water levels. Florida Scient. 50:49–61.

SMITH, R. B. AND D. R. BREININGER. 1995. Wading bird populations of the Kennedy Space Center. Bull. Mar. Sci. 57:230–236.

SWAIN, H. M. AND J. SHENKER. 1993. Waterbird use of rotationally managed saltmarsh impoundments. Final Report for St. Lucie Mosquito Control District, Ft. Pierce, FL.

———, D. R. BREININGER, D. S. BUSBY, K. B. CLARK, S. B. COOK, R. A. DAY, D. E. DEFREESE, R. G. GILMORE, A. HART, C. R. HINKLE, D. A. MCARDLE, P. M. MIKKELSEN, W. G. NELSON, AND A. J. ZAHORCAK. 1995. Introduction: Indian River Lagoon Biodiversity Conference. Bull. Mar. Sci. 57:1–7.

TAYLOR, D. S. 1989. The Sykes Creek impoundments: a massive, multi-use mitigation plan. J. Florida Anti-Mosq. Assoc. 60:23–27.

———, G. R. POULAKIS, S. R. KUPSCHUS, AND C. H. FAUNCE. 1998. Estuarine reconnection of an impounded mangrove salt-marsh in the Indian River Lagoon: short-term changes in fish fauna. Mangr. Saltmarsh. 2:29–36.

TROST, C. H. 1968. Study of wildlife usage of saltmarsh on the east coast of Florida before and after impoundment for mosquito and sandfly control. Unpub. final report: Bureau of Sport Fisheries and Wildlife, U.S.F.W.S. contract #14-16-0008-623, Florida State Board of Health, Entomological Research Center, Vero Beach, FL.

WEINSTEIN, W. P. 1979. Shallow marsh habitats as primary nurseries for fishes and shellfish, Cape Fear, North Carolina. Fish. Bull., US 77:339–357.

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REPTILE SURVEYS OF PINE ROCKLAND HABITAT IN SIX MIAMI-DADE COUNTY PARKS

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ABSTRACT: *We surveyed the reptile fauna of pine rockland habitat in six Miami-Dade County parks using quadrat sampling in 1996–97 and time-constrained sampling in 2001. Time-constrained sampling detected similar numbers of reptile species per park as quadrat sampling in one-quarter as much time. We detected only 7–8 reptile species per park regardless of park size, which ranged from 2.4 to 80.9 ha. Of 15 reptile species found using both survey methods, five are nonnative. The most frequently observed species were the nonnative brown anole (*Anolis sagrei*) and tropical house gecko (*Hemidactylus mabouia*), whose population apparently has increased during the five years between surveys. Pine rockland has a higher proportion of native to nonnative reptile species than human-modified habitats in Miami-Dade County. Standing and fallen dead South Florida slash pines (*Pinus elliottii *var.* densa*) provide important refugia for many rockland reptile species, but these microhabitats are often destroyed by the periodic fires that prevent succession to tropical hammock. Additional survey methods are necessary to detect uncommon and widely dispersed species, such as large snakes.*

Key Words: Reptiles, pine rockland, exotic species, sampling techniques, Florida

PINE rockland habitat occurs along the large outcropping of oolitic Miami limestone that extends from Miami through Homestead to the Long Pine Key area of Everglades National Park. The Miami Rock Ridge is 6–16 km wide and mostly 2–7 m above sea level (Snyder et al., 1990). The ridge has a very irregular surface; pinnacle rock (weathered oolitic limestone) and solution holes are common, and sandy soil is primarily restricted to occasional shallow depressions in the surface rock. In the Miami area, ca. 97% of pine rockland has been destroyed since European settlement, and the highly fragmented remaining patches are embedded in a matrix of agricultural and residential landscapes (O'Brien, 1998). Only 14% of the 420 pine rockland fragments (1524 ha) outside of Everglades National Park are in public ownership (O'Brien, 1998). Some of the largest and most intact tracts of pine rockland are under the jurisdiction of Metro Dade County Parks and Recreation Department.

Dalrymple (1988) surveyed pine rockland in Everglades National Park using drift fences, but no similar survey has been conducted in the highly fragmented pine rockland fragments outside the park. Because of surface oolitic limestone, installation of drift fences and pitfall traps is difficult in rockland habitat. A less labor-intensive method is needed to inventory the herpetofauna of rockland tracts. The original purpose of this study was to assess the effectiveness of quadrat sampling at detecting the state-threatened rim rock crowned snake (*Tantilla oolitica*) in rockland habitat in six Miami-Dade County parks. After quadrat sampling proved unsuccessful, we decided to compare the effectiveness of time-constrained sampling versus quadrat sampling at detecting reptile species in pine rockland in these six parks. Both sampling methods have set time limits, but quadrat sampling involves intensively searching a circumscribed area, whereas time-constrained sampling is restricted only by the boundaries of the habitat or study area (Campbell and Christman, 1982).

DESCRIPTION OF THE STUDY AREA—The study was conducted in pine rockland in six disjunct Miami-Dade County parks, with two parks in each of three size classes: ≤4 ha, 10–20 ha, and >40 ha. All parks are situated in southern Miami-Dade County between Kendall and Cutler Ridge. The two smallest parks are Pine Shore Preserve (2.4 ha) at SW 128 Street and SW 112 Avenue, and Ned Glenn Preserve (4.0 ha) at SW 188 Street and SW 87 Avenue. The medium-sized parks are the Tamiami Complex Addition Preserve (= Tamiami Complex; 10.5 ha) at SW 134 Street and SW 122 Avenue, and Rockdale Preserve (16 ha) between SW 144 Street and SW 152 Street just west of U.S. Highway 1. The largest parks are the Charles Deering Estate at Cutler (= Deering Estate; 46.5 ha) north of SW 167 Street and east of SW 72 Avenue, and the Larry and Penny Thompson Memorial Park (= Thompson Memorial Park; 80.9 ha) north of SW 184 Street and east of SW 134 Avenue. We surveyed pinelands at the north end of Deering Estate south of SW 152 Street, and the south end of Thompson Memorial Park south of SW 182 Terrace.

Pine rockland typically consists of an open-canopied forest of South Florida slash pine (*Pinus elliottii* var. *densa*) with a patchy understory of tropical and temperate shrubs and palms, including saw palmetto (*Serenoa repens*), cabbage palm (*Sabal palmetto*), poisonwood (*Metopium toxiferum*), winged sumac (*Rhus copallina*), rough velvet seed (*Guettarda scabra*), myrsine (*Myrsine floridana*), live oak (*Quercus virginiana*), and marlberry (*Ardisia escallonioides*). Ground cover is variable and often includes broomsedge (*Andropogon* spp.), wiregrass (*Aristida* spp.), muhly grass (*Muhlenbergia* spp.), rattlebox (*Crotalaria* spp.), partridge pea (*Cassia fasciculata*), coontie (*Zamia pumila*), and pinefern (*Anemia adiantifolia*) (Florida Natural Areas Inventory, 1990; Snyder et al., 1990).

Canopy closure by trees (mostly young slash pines) ≥5 m tall is 15–25% at all parks except Ned Glenn Preserve and Thompson Memorial Park, which have <5% canopy closure. Shrub cover (i.e., woody vegetation 0.5–5 m tall) is ≥50% at all parks. Exposed oolitic limestone covers ca. 20–30% of the surface in all parks except Ned Glenn Preserve and Thompson Memorial Park, which have ≥40% exposed surface limestone. Most parks have some areas with ≥75% exposed surface limestone. Anthropogenic debris was observed at all sites except Deering Estate and Thompson Memorial Park.

Relatively few large pine trees in the parks survived Hurricane Andrew, which struck Miami-Dade County on 24 August 1992 (Loope et al., 1994). Many standing pine snags (i.e., dead trees) with bark were present during surveys in 1996–97, but many had fallen by 2002. Due to subsequent fires and decomposition, remaining snags typically lacked bark and often consisted of only heartwood cores. Ned Glenn Preserve, Rockdale Preserve, and Thompson Memorial Park had few snags with rotten wood or loose bark suitable for sampling in 2002. In 2000, Ned Glenn Preserve was completely burned by two wildfires, and 4.5 ha of the area sampled in Thompson Memorial Park and 1.2 ha of Rockdale Preserve were burned by wildfires (Hazelton, 2002). In 2000–01, three wildfires burned 1.5 ha of Pine Shore Preserve (Hazelton, 2002). The portion of Deering Estate sampled was last burned in 1989 (Warren-Bradley, 2002), and Tamiami Complex has apparently not burned recently.

METHODS—Two randomly located quadrats (1000 m^2) were established in each park and sampled in September 1996 and May 1997 by two to four persons. Four man-hours of sampling was conducted per quadrat during each visit for a total of 96 man-hr of quadrat sampling. Sampling consisted of searching for reptiles by turning rocks, logs, and other debris; inspecting solution holes; raking through debris, soil, and rock rubble with a potato rake; and peeling loose bark off pine snags. During the second site visit, quadrats were moved $\geq$25 m away from the original quadrats because some types of refugia, particularly loose bark on snags, were destroyed during the first visit.

Time-constrained sampling was conducted in May 2002 by two persons in the same vicinity as the previous quadrats. Ned Glenn Preserve, however, was sampled in September 2002. Voucher specimens of nonnative herpetofauna were collected and deposited in the Florida Museum of Natural History (FLMNH), University of Florida (UF collection). One person used a "stump-ripper" snake hook, while the other person used a blowgun that shot tapered corks. Four man-hr were spent sampling each park; thus, only one-quarter as much effort was expended as in quadrat sampling. Time-constrained sampling involved similar searching techniques as quadrat sampling, but less surface debris was raked and fewer rocks turned. We were able to search most snags and fallen logs in all but the two largest parks, Deering Estate and Thompson Memorial Park, during the 4 man-hr of time-constrained sampling.

We decided to include only reptile sightings when comparing the effectiveness of different survey techniques, because amphibian presence and abundance are usually dependent upon suitable precipitation and nearby breeding sites. Suitable wetlands for reproduction of native amphibian species are not present near most of the rockland tracts sampled.

RESULTS—Combining both months of sampling, 5–6 reptile species were found per site using quadrat sampling (Table 1). Combining all six sites, 10 reptile species were recorded on quadrats, excluding a Florida box turtle (*Terrapene carolina bauri*) shell and shed snake skins (Table 1). During limited sampling outside quadrats, we detected three additional reptile species—scarlet kingsnake (*Lampropeltis triangulum elapsoides*), rough green snake (*Opheodrys aestivus*), and eastern diamondback rattlesnake (*Crotalus adamanteus*)—that suggested the possible efficacy of time-constrained sampling in pine rockland.

Time-constrained sampling five years later yielded 3–7 reptile species per site and a total of 12 reptile species for all six sites combined, excluding a box turtle shell, active gopher tortoise (*Gopherus polyphemus*) burrow, and shed diamondback rattlesnake skin (Table 1). The bark anole (*Anolis distichus*), reef gecko (*Sphaerodactylus notatus*), and ground skink (*Scincella lateralis*) were detected only during time-constrained sampling (Table 1). The mean number of reptile species

TABLE 1. Reptile species observed on quadrats in September 1996 and May 1997 and during time-constrained sampling in May or September 2002 of pine rockland in six Miami-Dade County parks, Florida. Numbers in parentheses represent shed snake skins, active tortoise burrows, or unhatched gecko eggs; X's represent gecko eggs observed but not counted.

Species	Pine Shore	Ned Glenn	Tamiami Complex	Rockdale	Deering Estate	Thompson Memorial
Turtles						
Gopherus polyphemus	0	0	0	0	1(2)	0
Sep 96/May 97	0/0	0/0	0/0	0/0	(1)/1	0/0
May or Sep 02	0	0	0	0	(1)	0
Terrapene carolina shell	0	0	2	0	0	0
Sep 96/May 97	0/0	0/0	0/1	0/0	0/0	0/0
May or Sep 02	0	0	1	0	0	0
Lizards						
Anolis carolinensis	13	4	1	2	0	8
Sep 96/May 97	6/5	2/2	1/0	1/1	0/0	3/1
May or Sep 02	2	0	0	0	0	4
Anolis distichus	0	0	0	0	2	0
Sep 96/May 97	0/0	0/0	0/0	0/0	0/0	0/0
May or Sep 02	0	0	0	0	2	0
Anolis sagrei	33	13	46	44	43	35
Sep 96/May 97	8/8	2/2	18/17	17/10	18/3	1/9
May or Sep 02	17	9	11	17	22	25
Eumeces inexpectatus	5	10	3	2	5	4
Sep 96/May 97	3/0	0/3	0/0	1/1	0/2	2/1
May or Sep 02	2	7	3	0	3	1
Hemidactylus garnotii	8	2	3(16)	5(2)	7(8)	3
Sep 96/May 97	3/5	0/2	0/2	2/3	5/0	1/2
May or Sep 02[1]	0(0)	0	1(16)	0(2)	2(8)	0(0)
Hemidactylus mabouia	17(5)	8	11(8)	18(11)	0	22(1)
Sep 96/May 97	0/0	0/2	0/0	0/0	0/0	0/0
May or Sep 02[1]	17(5)	6	11(8)	18(11)	0	22(1)
Hemidactylus unhatched eggs	24	0	>2	9	>2	11
Sep 96/May 97	7/17	0/0	X/1	6/3	2/X	2/6
May or Sep 02	0	0	1	0	0	3
Scincella lateralis	0	1	0	0	0	0
Sep 96/May 97	0/0	0/0	0/0	0/0	0/0	0/0
May or Sep 02	0	1	0	0	0	1
Sphaerodactylus notatus	0	0	0	0	1(2)	0
Sep 96/May 97	0/0	0/0	0/0	0/0	0/0	0/0
May or Sep 02[1]	0	0	0	0	1(2)	0
Snakes						
Coluber constrictor	0	(1)	0	0	2	0
Sep 96/May 97	0/0	0/(1)	0/0	0/0	0/0	0/0
May or Sep 02	0	0	0	0	2	0
Crotalus adamanteus	0	0	(1)	0	0	0
Sep 96/May 97	0/0	0/0	0/0	0/0	0/0	0/0
May or Sep 02	0	0	(1)	0	0	0
Diadophis punctatus	0	0	0	1	0	0
Sep 96/May 97	0/0	0/0	0/0	0/1	0/0	0/0
May or Sep 02	0	0	0	0	0	0

TABLE 1. Continued.

Species	Pine Shore	Ned Glenn	Tamiami Complex	Rockdale	Deering Estate	Thompson Memorial
Elaphe guttata	0	0	5	0	0	(1)
Sep 96/May 97	0/0	0/0	1/0	0/0	0/0	0/0
May or Sep 02	0	0	4(7)	0	0	(1)
Ramphotyphlops braminus	2	0	1	4	2	1
Sep 96/May 97	1/1	0/0	1/0	3/0	1/1	1/0
May or Sep 02	0	0	0	1	0	0
Unidentified snake skin	(1)	0	(1)	(1)	0	(1)
Sep/May 97	(1)/0	0/0	(1)/0	0/(1)	0/0	0/(1)
May or Sep 02	0	0	0	0	0	0
Total[2]	78	38	70	76	63	73
August 96/May 97	21/19	4/11	21/19	24/16	24/7	8/13
May or Sep 02	38	23	30	36	32	52
No. species[3]	7	7	9	7	8	7
August 96/May 97	5/4	2/5	4/3[4]	5/4	4/4	5/4[5]
May or Sep 02	4	4	7	3	7	5

[1] Eggs were incubated to determine species.
[2] Excludes eggs, burrows, turtle shells, and shed skins.
[3] Includes eggs, burrows, turtle shells, and shed skins but does not include off-quadrat species.
[4] *Lampropeltis triangulum* and *Crotalus adamanteus* were recorded off quadrats in May 1997.
[5] *Opheodrys aestivus* was recorded off quadrats in September 1996.

detected per site did not differ using both methods ($t = 0.70$, df $= 10$, $P = 0.50$), but time-constrained sampling required one-quarter as much effort and covered a larger area than quadrat sampling.

In the three size classes of parks, we detected a mean of 7.0 or 8.0 reptile species after combining the results of both sampling methods and including wildlife sign. Although both methods appeared to be reasonably successful at detecting common species, they were ineffective at detecting uncommon or widely dispersed species, such as large snakes. We did not find any rim rock crowned snakes. Five of the 15 reptile species detected in at least one park are nonnative: bark anole, brown anole (*Anolis sagrei*), Indo-Pacific gecko (*Hemidactylus garnotii*), tropical house gecko (*H. mabouia*), and Brahminy blind snake (*Ramphotyphlops braminus*) (Table 1).

Eighty-nine percent of 225 anoles (*Anolis* spp.) were observed on the ground, on bushes or palmetto fronds, on fallen pine logs, or on trunks of snags (Table 2). The proportion of anoles observed in these four microhabitats differed significantly between sampling methods ($\chi^2 = 28.6$, df $= 3$, $P < 0.0001$), but some of this difference could be due to habitat differences between years. Seventy-one percent of 28 southeastern five-lined skinks (*Eumeces inexpectatus*) were found under pine logs, under rocks or other objects, under bark on snags, or inside the wood of snags (Table 2). All Brahminy blind snakes except one were found in, under, or immediately adjacent to a fallen log or snag (Table 2); two individuals were found in September 1996 under the bark on pine snags ca. 1 m above the ground. All five corn snakes (*Elaphe guttata*) were found inside the wood of pine snags.

TABLE 2. Number of individuals of the most common reptiles observed in various microhabitats during quadrat searches (QS) in September 1996 and May 1997 and during time-constrained sampling (TCS) in May or September 2002 of pine rockland in six Miami-Dade County parks, Florida.

	Anolis spp.		*Hemidactylus* spp.		*Eumeces inexpectatus*		*Ramphotyphlops braminus*	
Microhabitat	QS	TCS	QS	TCS	QS	TCS	QS	TCS
On ground or rock	22	22	0	0	1	0	0	0
On trunk of live pine	4	3	0	0	0	0	0	0
On trunk of snag	11	29	0	0	0	2	0	0
On bush or palmetto frond	38	25	0	0	0	0	0	0
On fallen log	44	10	0	0	0	1	0	0
On wooden fence	0	4	0	2	0	0	0	0
Under fallen pine log	2	0	0	0	3	0	1	1
Under rock or other object	0	1	1	1	2	2	1	0
Under bark on pine log	0	0	5	0	1	0	2	0
Under bark on snag	3	4	9	18	2	2	1	0
Inside fallen pine log	0	0	1	2	1	2	1	0
Inside wood of snag	0	3	10	52	2	7	2	0
In soil at base of snag	0	0	0	1	0	0	1	0
Total	124	101	26	76	12	16	9	1

Eighty-three percent of 102 geckos (*Hemidactylus* spp.) were found under the bark or inside the wood of snags. Gecko eggs were found in similar microhabitats as adults. Eggs collected during the time-constrained survey hatched 0–39 days later and yielded valuable species occurrence information, such as the presence of the Indo-Pacific gecko at Rockdale Preserve. We observed only one Indo-Pacific gecko in 2002 at Tamiami Complex but collected 16 eggs. At times, we were unable to capture a gecko, and visual identification of species was sometimes problematic.

DISCUSSION—During quadrat searches, we found an average of 5.2 reptile species per site and 10 species overall, excluding wildlife sign (i.e., shed skins and gopher tortoise burrows). During time-constrained sampling, we found 4.5 reptile species per site and 11 species overall in one-quarter as much time and during only one sampling period. Our comparisons of the effectiveness of the two sampling methods are complicated by possible changes over the five-year period in the number of species, relative abundance of species, or searchable microhabitats at various sites. Also, different observers were used during our surveys, which only had one observer (KME) in common. The only previous study in Florida that compared these two sampling methods was conducted in eight different habitats or sites in Marion County (Florida Game and Fresh Water Fish Commission, 1976). This earlier study found time-constrained sampling (average of 7.0 reptile species per site) to be more effective than quadrat sampling (3.7 reptile species per site), but only half as much time was spent searching quadrats (2 man-hr per 1000-m^2 quadrat) as during our study.

Our results suggest that time-constrained sampling is a more efficient method for surveying reptiles in pine rockland than quadrat sampling, but some of the observed differences may have been due to changes in reptile communities or habitats between years. Additional simultaneous comparisons of the two sampling methods using the same observers are necessary before definitive conclusions can be drawn. The use of additional survey methods would help compile a more complete species list. Drift fences with funnel traps are effective at trapping a variety of herpetofaunal species (e.g., Dalrymple, 1988; O'Hare and Dalrymple, 1997; Enge, 2001), but are difficult to install in rockland because of surface limestone and shallow soil. In large rockland tracts intersected by roads, vehicular surveys would probably detect additional species, particularly snakes (Dalrymple et al., 1991). Time-constrained sampling would probably have been more effective if both persons had been equipped with snake hooks for turning objects, searching snags and logs, and raking through debris. The person with the blowgun could not easily conduct some microhabitat searches without a snake hook.

Geckos, southeastern five-lined skinks, and some snake species hide primarily in pine snags and logs, which are often destroyed by fire. Suitable pine snags for sheltering reptiles were numerous when quadrat sampling was conducted because of the effects of Hurricane Andrew, and 15 reptiles were found under bark and 15 reptiles inside the wood of snags (Table 2). However, suitable snags were less abundant during time-constrained sampling because wildfires in 2000 at three of the parks destroyed much of the above-ground deadwood microhabitats. Relatively few pine snags retained bark, and most reptiles sheltered instead in those snags with rotten wood that had not burned completely, which explains why we found 66 reptiles inside snags but only 24 reptiles under bark during time-constrained sampling (Table 2). We found corn snakes only inside pine snags but suspect they would have also been found under the bark of snags, if more snags had had bark or if air temperatures had been cooler. Although fire destroys some refugia, it sometimes kills pine trees and creates new refugia. Periodic fires, which probably occurred every 3–10 years during pre-Columbian times, are necessary to prevent succession of pinelands to tropical hammock, which may take only 20–30 years on the mainland in southern Florida (Robertson, 1953; Alexander, 1967; Hofstetter, 1984). If understory development progresses longer than 8–10 years in pinelands, fires are precluded or catastrophic (Robertson, 1954; Loope and Dunevitz, 1981; Snyder, 1986).

We might have been less successful at detecting small, secretive snake species, such as the southern ringneck (*Diadophis p. punctatus*) and Brahminy blind snake (theoretically, also the rim rock crowned snake), during time-constrained sampling than during quadrat sampling because we spent less time turning rocks and raking through the fire-depleted debris. Finding Brahminy blind snakes above the ground in snags corroborates observations of arboreal activity in this species (Das and Wallach, 1998). Several explanations might account for discrepancies in observations of anoles in various microhabitats using the two methods. During time-constrained sampling, we might have been looking farther ahead and been more prone to detect anoles on the trunks of pine snags before they either ascended out of sight or descended to the ground. The much slower and more intensive quadrat sampling might have resulted in better detection of anoles on bushes, palmetto fronds, and fallen pine logs. We also suspect that one observer (KLK) used only during time-constrained sampling was better at spotting anoles from afar.

A drift-fence survey in pine rockland in Everglades National Park recorded 13 reptile species, all native, with green anoles predominating (Dalrymple, 1988). Species detected in Everglades National Park but not in the six parks we surveyed were the striped mud turtle (*Kinosternon baurii*), island glass lizard (*Ophisaurus compressus*), eastern indigo snake (*Drymarchon corais couperi*), dusky pigmy rattlesnake (*Sistrurus miliarius barbouri*), peninsula ribbon snake (*Thamnophis sauritus sackenii*), and eastern garter snake (*T. s. sirtalis*). The paucity of nearby wetlands and scarcity of anurans in the county parks surveyed probably account for our failure to find the latter three snake species, which prey heavily on anurans. We detected the following native species that were not trapped in pinelands in Everglades National Park: gopher tortoise (Deering Estate), rough green snake (off-quadrat in Thompson Memorial Park), scarlet kingsnake (off-quadrat in Tamiami Complex), and diamondback rattlesnake (Tamiami Complex). The relatively small tracts of pine rockland contained in Miami-Dade County parks are more depauperate than similar habitat in Everglades National Park, but even the smallest tracts support populations of some species, particularly nonnative lizards. Fewer individuals and species of herpetofauna were trapped in pinelands than in wet prairies or hammocks in Everglades National Park, and the presence of many species in pinelands is dependent upon interdigitating prairies and scattered hammocks (Dalrymple, 1988). Wet prairies are absent in all the county parks we surveyed, and intact hammocks are present only at Deering Estate. We suspect that the ringneck snake, corn snake, and Everglades racer (*Coluber constrictor paludicola*) are present in most of the county parks surveyed, and the primary prey of the latter two species is probably nonnative lizard species.

Upland snake species with large home ranges have undergone severe population reductions in southern Florida (Wilson and Porras, 1983). The largest pineland surveyed, Thompson Memorial Park, is adjacent to extensive pinelands on Metrozoo property. Metrozoo staff have observed only one indigo snake and one diamondback rattlesnake during the past six years in the zoo's ca. 178 ha of undeveloped (mostly pinelands) and 121 ha of developed areas, but frequently they have observed racers, eastern coachwhips (*Masticophis f. flagellum*), and ringneck snakes (Conners, 2002). We observed none of these species during either quadrat or time-constrained sampling in a 30-ha portion of Thompson Memorial Park, although some of these species are undoubtedly present. We were surprised to find a diamondback rattlesnake in Tamiami Complex, one of the smaller preserves, both in 1997 and 2002 (shed skin), and we suspect that the two sightings were not of the same individual. The total amount of suitable diamondback rattlesnake habitat remaining in the area is less than that of the smallest home range size (25.7 ha) found for an individual rattlesnake in northern Florida (Timmerman, 1995). Potentially suitable rattlesnake habitat includes 10.5 ha in the preserve, 7 ha of nearby pinelands, and a mown powerline right-of-way with very little cover. The available habitat is surrounded by a warehouse complex, subdivision, plant nursery, and the Florida Turnpike.

Our findings suggest that the gecko community has changed during the five years between surveys. In 1997, we found two tropical house geckos only in Ned Glenn Preserve. In 2002, however, we found 71 tropical house geckos in five parks (Table 1) and incidentally observed one that was diurnally active in the garden portion of the sixth park, Deering Estate. In 1997, we found 29 Indo-Pacific geckos in five parks, whereas in 2002, we found only four individuals in three parks. Meshaka and co-workers (1994) suggested that the tropical house gecko probably occurred in Florida prior to its documentation in 1990 in the upper Florida Keys, Monroe County (Lawson et al., 1991), and it was probably introduced at multiple sites in Miami and the Florida Keys via imported commercial plants. The tropical house gecko is rapidly expanding its range and has already replaced the longer established Indo-Pacific gecko on buildings in some areas (Meshaka, 2000), and we suspect that the same replacement process is occurring in Miami-Dade pinelands.

Conservatively, at least 24 nonnative reptile species currently have established populations in Miami-Dade County. In the future, species in South Florida that are habitat-specific and have limited ranges will likely become endangered, extirpated, or extinct as their habitat is destroyed, and populations of native and exotic species that are adapted to disturbed, urban, or agricultural environments will increase (Forys and Allen, 1999). Of all reptile species or sign observed in all six parks combined, 12 species are native and five are exotic. In contrast, only three native and seven exotic reptile species were found during a survey of the Doc Thomas House, a 1.4-ha tract of seminatural pineland/hammock habitats in South Miami, Miami-Dade County (Meshaka, 1999a). Four native and eight exotic reptile species were found at the Kampong, a 4-ha botanical garden in Coconut Grove, Miami-Dade County (Meshaka, 1999b). This suggests that the presence of pine rockland tracts, particularly large ones, in the urban landscape of Miami-Dade County might ensure the continued survival of some native reptile species in an area that is increasingly dominated by exotic species. Several exotic reptile species, such as the bark anole, knight anole (*Anolis equestris*), and Tokay gecko (*Gekko gecko*), are common in the surrounding human-modified landscape but apparently seldom invade remnant pockets of pine rockland.

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LITERATURE CITED

ALEXANDER, T. R. 1967. A tropical hammock on the Miami (Florida) limestone—a twenty-five year study. Ecology 48:863–867.

CAMPBELL, H. W. AND S. P. CHRISTMAN. 1982. Field techniques for herpetofaunal community analysis. Pp. 193–200. *In*: SCOTT, N. J., JR. (ed.). Herpetological Communities. U.S. Fish and Wildl. Serv. Wildl. Res. Rept. No. 13.

CONNERS, S. 2002. General Curator, Miami Metrozoo, Miami, FL. Pers. Comm.

Dalrymple, G. H. 1988. The herpetofauna of Long Pine Key, Everglades National Park, in relation to vegetation and hydrology. Pp. 72–86. *In*: Szaro, R. C., K. E. Severson, and D. R. Patton (tech. coords.). Proceedings of a symposium on the management of reptiles, amphibians, and small mammals in North America. U.S. For. Serv. Gen. Tech. Rept. RM-166.

———, F. S. Bernardino, Jr., T. M. Steiner, and R. J. Nodell. 1991. Patterns of species diversity of snake community assemblages, with data on two Everglades snake assemblages. Copeia 1991: 517–521.

Das, I. and V. Wallach. 1998. Scolecophidian arboreality revisited. Herpetol. Rev. 29:15–16.

Enge, K. M. 2001. The pitfalls of pitfall traps. J. Herpetol. 35:467–478.

Florida Game and Fresh Water Fish Commission. 1976. Cross Florida Barge Canal restudy report: Wildlife study. Vol. II, Append. B: Herpetology study. Dept. Army Corps Engineers, Jacksonville, FL. 217 pp.

Florida Natural Areas Inventory. 1990. Guide to the Natural Communities of Florida. Florida Nat. Areas Inventory and Florida Dept. Nat. Resour., Tallahassee. 111 pp.

Forys, E. A. and C. R. Allen. 1999. Biological invasions and deletions: community change in South Florida. Biol. Conserv. 87:341–347.

Hazelton, D. 2002. Nat. Areas Manage., Miami-Dade Co. Park and Recreation Dept., Miami, FL. Pers. Comm.

Hofstetter, R. H. 1984. The effect of fire on the pineland and sawgrass communities of southern Florida. Pp. 465–476. *In*: Gleason, P. J. (ed.). Environments of South Florida: Present and Past II. Miami Geol. Soc., Coral Gables, FL.

Lawson, R., P. G. Frank, and D. L. Martin. 1991. A gecko new to the United States herpetofauna, with notes on geckoes of the Florida Keys. Herpetol. Rev. 22:11–12.

Loope, L. L. and V. L. Dunevitz. 1981. Impact of fire exclusion and invasion of *Schinus terebinthifolius* on limestone rockland pine forests of southeastern Florida. South Florida Res. Cent. Rept. T-645, Everglades National Park, Homestead, FL. 30 pp.

———, M. Duever, A. Herndon, J. Snyder, and D. Jansen. 1994. Hurricane impact on uplands and freshwater swamp forest. BioScience 44:238–246.

Meshaka, W. E., Jr. 1999a. The herpetofauna of the Doc Thomas House in South Miami, Florida. Florida Field Nat. 27:121–123.

———. 1999b. The herpetofauna of the Kampong. Florida Scient. 62:153–157.

———. 2000. Colonization dynamics of two exotic geckos (*Hemidactylus garnotii* and *H. mabouia*) in Everglades National Park. J. Herpetol. 34:163–168.

———, B. P. Butterfield, and B. Hauge. 1994. *Hemidactylus mabouia* as an established member of the Florida herpetofauna. Herpetol. Rev. 25:80–81.

O'Brien, J. J. 1998. The distribution and habitat preferences of rare *Galactia* species (Fabaceae) and *Chamaesyce deltoidea* subspecies (Euphorbiaceae) native to southern Florida pine rockland. Nat. Areas J. 18:208–222.

O'Hare, N. K. and G. H. Dalrymple. 1997. Wildlife in southern Everglades wetlands invaded by melaleuca (*Melaleuca quinquenervia*). Bull. Florida Mus. Nat. Hist. 41:1–68.

Robertson, W. B., Jr. 1953. A survey of the effects of fire in Everglades National Park. Natl. Park Serv., Homestead, FL. 169 pp.

———. 1954. Everglades fires—past, present, and future. Everglades Nat. Hist. 2:11–16.

Snyder, J. R. 1986. The impact of wet season and dry season prescribed fires on Miami Rock Ridge pineland, Everglades National Park. South Florida Res. Cent. Rept. SFRC-86/06, Everglades National Park, Homestead, FL. 106 pp.

———, A. Herndon, and W. B. Robertson, Jr. 1990. South Florida rockland. Pp. 230–277. *In*: Myers, R. L. and J. J. Ewel (eds.). Ecosystems of Florida. Univ. Central Florida Press, Orlando, FL.

Timmerman, W. W. 1995. Home range, habitat use, and behavior of the eastern diamondback rattlesnake (*Crotalus adamanteus*) on the Ordway Preserve. Bull. Florida Mus. Nat. Hist. 38, Part I(5):127–158.

WARREN-BRADLEY, A. 2002. Charles Deering Estate at Cutler, Miami-Dade County Park and Recreation Dept., Miami, FL. Pers. Comm.

WILSON, L. D. AND L. PORRAS. 1983. The ecological impact of man on the South Florida herpetofauna. Univ. Kansas Mus. Nat. Hist. Spec. Publ. No. 9. 89 pp.

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BATS OF THE SUB-TROPICAL CLIMATE OF MARTIN AND ST. LUCIE COUNTIES, SOUTHEAST FLORIDA

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Abstract: *Surveys for bats using mist nets and acoustic detectors were conducted in Martin and St. Lucie Counties, and the extreme northern section of Palm Beach County, Florida from 2000–2002. Mist nets were set on 36 nights for a total of 360.0 total net hours, with an overall capture rate of 0.19 bats/net hour. Five species of bats were captured including evening bats (Nycticeius humeralis, n = 36), Brazilian free-tailed bats (Tadarida brasiliensis; n = 17), Seminole bats (Lasiurus seminolus; n = 10), northern yellow bats (Lasiurus intermedius; n = 3), and eastern pipistrelles (Pipistrellus subflavus; n = 2). Bats were captured in all natural communities, but higher capture rates were recorded in ruderal/disturbed habitat. Evening and Seminole bats were captured at a greater frequency in natural areas, while Brazilian free-tailed bats were primarily associated with ruderal areas near structures. Acoustic detections during the survey indicated a higher activity rate in open, early successional or ruderal habitats than indicated by capture success. Bat activity was documented throughout the year, though a limited winter sampling period was conducted.*

Key Words: Bats, southeast Florida, natural communities

Bat species richness increases with the abundance and diversity of roost sites (Humphrey, 1975). Sources of freshwater for drinking may also be important to maintain bat populations (Jennings, 1958). Additionally, a mosaic of habitats that includes roost sites, alternate roost sites, foraging and drinking habitat of bats may be required to protect bat populations (Ball, 2002). Because south Florida has low topographic relief and is typically hydric in character, it has no terrestrial caves and all bats in south Florida use foliage, tree cavities, or tree boles for roost sites (Barbour and Davis, 1969). Some species such as the Brazilian free-tailed bat and evening bat have adapted to roost in anthropogenic structures such as attic lattices, eaves, and roofs.

Four species of bats (*Lasiurus intermedius*, *L. seminolus*, *Nycticeius humeralis*, and *Tadarida brasiliensis*) are known from Martin and St. Lucie Counties during recent times (Barbour and Davis, 1969). No species of bat known to exist in any area of south Florida is federally listed as threatened or endangered. However, the Wagner's mastiff bat (*Eumops glaucinus*) has the most limited distribution of any bat in the eastern United States and is listed by the State of Florida as endangered. This rare species has not been documented from Martin or St. Lucie Counties in recent times. Fossil records indicate *E. glaucinus* occurred north of St. Lucie County within the past 10,000 years (Morgan, 1991) and has been recently documented in pine flatwoods of southwest Florida indicating the range of this species is not well understood (Bellwood, 1981). Forested sites, no matter how fragmented or isolated, within high-density urban areas may provide the last remnants of adequate roost sites for cavity and foliage roosting bats in southeast Florida.

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Information on the status of bats is lacking for many areas of Florida and southern Florida in particular. As the human population growth continues to expand in Martin and St. Lucie Counties, this will result in the loss of habitat for not only bats but all other species of wildlife. In two surveys of bats in south Florida, bats were assessed to be rare or uncommon (Jennings, 1958; Robson, 1989). In coastal scrub with canopy cover, evening and Seminole bats were determined to be common but the survey was limited to a small 400 m transect (Hutchinson, 2001). In this paper, information is presented on the communities of bats that inhabit the coastal section of Martin and St. Lucie Counties in southeast Florida.

METHODS—Surveys were conducted along the Atlantic Coastal Ridge and adjacent low-lying flatwoods, wet prairie communities in Jonathan Dickinson State Park (JDSP), Atlantic Ridge Preserve State Park (ARSP), Seabranch Preserve State Park (SEPSP), Avalon State Park (ASP) and Savannas Preserve State Park (SAPSP). The first three parks are located in Martin County, southeast Florida and occur within 3.2–16.0 km of the Atlantic Ocean. A small section of JDSP that includes part of the Loxahatchee River tributary lies in the extreme northern part of Palm Beach County. Avalon State Park and SPSP are located in Saint Lucie County and occur <5.0 km of the Atlantic Ocean.

Dominant natural communities in which surveys were conducted include: scrub, pine flatwoods, hardwood hammock, freshwater marsh, blackwater stream, and ruderal/disturbed habitat. At ASP, maritime hammock and estuarine tidal marsh were the dominant natural communities in which mist-nets were placed. Further descriptions of each natural community are described in Myers and Ewel (1990). For this survey, ruderal habitat is defined as habitat that exhibits <25% natural vegetation. It typically occurs in close proximity to undisturbed habitat, but has been altered by recreational areas, buildings, and drainage ditches and canals. The climate of the area is subtropical and temperatures seldom fall below 0°C. Average annual temperature and relative humidity are 23.3°C and 73%, respectively (Henry et al., 1994). Annual precipitation is ca. 152 cm per year with the highest average annual precipitation occurring in September (Henry et al., 1994).

Surveys were conducted from 5 May 2000 to 23 January 2002. Mist nets (6.0, 9.0 and 12.0 m; single and double stacked) were placed across streams, roads, trails, canopy gaps, road rut ponds, small constructed ponds, and perpendicular to structures. Several single nets were typically set over water while several double-stacked nets were placed along roads, trails, and canopy gaps. Modified variations of the net configurations suggested by Kunz and Kurta (1988) were utilized and each net (single or double-stacked) was counted in the number of net hours. Nets were raised at sunset and monitored for 2-4 hours. Nets were typically set for only one night at each location, but some sites were re-sampled at a later date. Captured bats were removed from the net and placed in a cotton bag. Bats were weighed to the nearest 0.5 g, sexed, and aged. Age (juvenile or mature) was determined based on the time of year, mass of the bat, and the presence of closure of the cartilaginous, epiphyseal growth plates in the finger bone (Anthony, 1988). Bat activity was also monitored acoustically using a QMC Mini-2 bat detector (Ultra Sound Advice, London, U.K.). Acoustic activity was monitored with the detector between the ranges of 20-40 Khz, the frequency range at which bats were most often detected. Detections were recorded at two-minute intervals to standardized detection rates of activity. Number of detections per hour was recorded as an index of activity for each site.

Means and standard error were calculated for capture and detection rates per hour among habitats and seasons. Comparisons based on capture and detection rates per hour were analyzed for differences among habitats and seasons using Kruskal-Wallis non-parametric test. Due to limited winter surveys, fall and winter surveys were combined for statistical analysis.

RESULTS—Mist nets were set on 36 nights for a total of 360.0 total net hours, with an overall capture rate of 0.19 bats/net hour (S.E. = 0.06). Sixty eight bats were captured representing five species including evening bats (*Nycticeius humeralis*, n = 36), Brazilian free-tailed bats (*Tadarida brasiliensis*; n = 17), Seminole bats (*Lasiurus seminolus*; n = 10), northern yellow bats (*Lasiurus intermedius*; n = 3), and eastern pipistrelles (*Pipistrellus subflavus*; n = 2).

Bats were captured in every park except ASP (Table 1). However, at ASP a northern yellow bat was observed flying near the nets. A disproportionate sampling effort prevented direct comparison of species diversity among parks. Surveys were concentrated in JDSP, the largest of the 5 parks surveyed, and all 5 species were captured.

Males and females of all five species were captured during the survey except for male eastern pipistrelles. Pregnant or lactating females of all species except the northern yellow bat were captured, suggesting that at least 4 of the 5 species captured breed in the area. Limited captures of northern yellow bats indicate this species may be rare in the area or only present in the area during the spring and fall, possibly migrating through the area. However, a juvenile female northern yellow bat was captured in the spring at JDSP indicating that northern yellow bats may breed in the area or migrate considerable distances once the maternity season is over.

Evening bats were the most frequently captured species, accounting for 53.0 % of all bats. Brazilian free-tailed bats were the next most frequently captured species at 25.0 %, which may be an under-representation on a landscape level because three known roost sites of >40 individuals were observed outside park boundaries near residential areas adjacent to SEPSP and SAPSP. Another population of >30 Brazilian free-tailed bats was excluded from a bathroom facility at JDSP in 1999 for safety concerns.

No significant difference was detected for bats among the habitats sampled for captures or detections (Captures: $H = 5.94$, d.f. = 5, $p = 0.31$; Detections: $H = 4.53$, d.f. = 5, $P = 0.48$), though sample sizes were limited. The highest capture rates were recorded in freshwater marsh and ruderal/disturbed habitat, while the highest detection rates were recorded in ruderal/disturbed and hardwood hammock habitat (Table 2). The majority of bats were captured in ruderal/disturbed habitat accounting for 58.8% of all captures (Table 3). Evening bats were captured in all habitats, while Brazilian free-tailed bats were only captured in ruderal/disturbed habitat. Seminole bats were captured in all habitats surveyed except hardwood hammock. Northern yellow bats were captured in hardwood hammock, blackwater stream, and ruderal habitat. Eastern pipistrelles were only captured over blackwater stream habitat.

Natural communities with the highest number of captures were mature scrub and blackwater stream at 14.7% and 13.2%, respectively. Four of the five species documented during the survey were captured in blackwater stream and ruderal/disturbed habitat. Only 3 bats (4.4 % of all captures) were captured in pine flatwoods, the most common natural habitat, within the survey area. Three bats were also captured in freshwater marsh habitat intermixed with pine flatwoods. Hardwood

TABLE 1. Species of bats captured at Florida State Parks surveyed along the Treasure Coast area of Martin and St. Lucie Counties in southeast Florida. Number in parenthesis indicates juveniles.

			Pipistrellus subflavus		*Lasiurus seminolus*		*Lasiurus intermedius*		*Nycticeius humeralis*		*Tadarida brasiliensis*	
Location	No. Net Nights (net hours)	Total Captures	M	F	M	F	M	F	M	F	M	F
JDSP	19 (177.0)	42		2 (1)	1 (0)	2 (1)	2 (1)	1 (1)	5 (0)	13 (2)	12 (1)	4 (0)
ARSP	1 (12.5)	10							6 (0)	3 (0)	1 (0)	
SEPSP	9 (89.5)	13			3 (1)	3 (1)			4 (0)	3 (1)		
ASP[a]	1 (7.5)	0										
SAPSP	6 (73.5)	3			1 (0)				1 (0)	1 (0)		
TOTAL	36 (360.0)		0 (0)	2 (1)	5 (1)	5 (2)	2 (1)	1 (1)	16 (0)	20 (3)	13 (1)	4 (0)

[a] *L. intermedius* observed flying at net.

TABLE 2. Capture, detection rate, and standard error of bats by habitat in Florida State Parks surveyed along Martin and St. Lucie Counties in southeast Florida.

Habitat	Captures / hr (S.E.)	Detections / hr (S.E.)
Blackwater Stream	0.13 (0.09)	8.17 (3.76)
Freshwater Marsh	0.44 (0.44)	5.07 (1.90)
Hardwood Hammock	0.05 (0.05)	11.63 (4.69)
Pine Flatwoods	0.10 (0.06)	5.83 (1.76)
Ruderal / Disturbed	0.43 (0.13)	11.79 (1.77)
Scrub	0.15 (0.07)	9.95 (4.20)

hammock, which is rare within the parks surveyed, also accounted for 4.4% (n = 3) of all captures.

Bats were captured during every season of the year though limited net nights (n = 2) were conducted during the winter months of December to February (Table 4). No significant difference was detected for capture rate per hour based on season, combining fall and winter surveys (Captures: H = 0.93, d.f. = 2, P = 0.63). The highest number of captures (39.7%) was recorded in the late spring when juvenile bats become volant.

Bats were acoustically detected during 35 of the 36 survey nights. Acoustic detection averaged 9.5 bat-passes/hr (S.E. = 1.36) for the entire survey period, but varied between 0.0 and 30.0 bat-passes/hr based on a 2-minute detection interval. The highest average acoustic detection rate for bats was in hardwood hammock (11.6 bat-passes/hr; SE = 4.69) and ruderal (11.8 bat-passes/hr; SE = 1.77). Acoustic detection rates were lowest in freshwater marsh and pine flatwoods at 5.1 bat-passes/hr (SE = 1.90) and 5.8 bat-passes/hr (SE = 1.76), respectively. Though limited surveys were conducted during the winter months (n = 2), acoustic detections were higher during the winter months (20.50 bat-passes/hr; SE = 9.5) compared to spring (10.21 bat-passes/hr; SE = 2.68), summer (8.64 bat-passes/hr; SE = 1.45), and fall (7.82 bat-passes/hr; SE = 3.29). Due to limited surveys during the winter, the fall and winter samples were combined for statistical analysis. No significant difference was detected for acoustic detections per hour based on season (Detections: H = 0.57, d.f. = 2, P = 0.753). The highest index of activity for a single night was recorded at a ruderal site in SAPSP and an early successional scrub at SEPSP where 30 bat-passes/hr were detected. Both sites were open with few trees, <25% canopy cover, and within 400 m of a freshwater marsh. No bats were captured at either site.

DISCUSSION—Mist-net captures and acoustic detection indicate that bats use a variety of habitats along the coastal areas of Martin and St. Lucie Counties. Based on capture rates, the evening and Seminole bats are the most frequently captured bats in natural communities along the Treasure Coast of Florida. Both of these species utilize natural roost sites that include foliage, cavities, and exfoliating bark (Menzel et al., 1998; 1999); the evening bat also utilizes human dwellings and other structures (Barbour and Davis, 1969). The Brazilian free-tailed bat was only documented in ruderal habitat, which may be a reflection of this species' tendency to

TABLE 3. Species of bats captured by habitat at Florida State Parks surveyed along the Treasure Coast area of Martin and St. Lucie Counties in southeast Florida.

Habitat	No. Net Nights (Net Hours)	*Pipistrellus subflavus*	*Lasiurus seminolus*	*Lasiurus intermedius*	*Nycticeius humeralis*	*Tadarida brasiliensis*	TOTAL
Scrub	6 (65.75)		5		5		10
Pine Flatwoods	4 (29.50)		1		2		3
Hardwood Hammock	6 (57.00)			1[a]	2		3
Freshwater Marsh	3 (34.75)		2		1		3
Blackwater Stream	7 (66.75)	2	1	1	5		9
Ruderal (disturbed)	10 (106.25)		1	1	21	17[b]	40
TOTAL	36 (360.00)	2	10	3	36	17	68

[a] *L. intermedius* observed flying at net.

[b] One *T. brasiliensis* captured in an isolated constructed pond in pine flatwoods.

TABLE 4. Species of bats captured by season at Florida State Parks surveyed along the Treasure Coast area of Martin and St. Lucie Counties in southeast Florida. Seasons were defined as Spring (March–May), Summer (June–August), Fall (September–November), and Winter (December–January).

Season	No. Net Nights (Net Hours)	*Pipistrellus subflavus*	*Lasiurus seminolus*	*Lasiurus intermedius*	*Nycticeius humeralis*	*Tadarida brasiliensis*	TOTAL
Spring	10 (90.5)		1	1	19	6	27
Summer	15 (142.5)	2	7		5	8	22
Fall	9 (104.0)			2[a]	9	3	14
Winter	2 (23.0)		2		3	[b]	5
TOTAL	36 (360.0)	2	10	3	36	17	68

[a] *L. intermedius* observed flying at net.

[b] No ruderal sites surveyed during winter.

roost in human made structures (Barbour and Davis, 1969). Discrepancies between capture success and acoustic detection during the survey period indicates that many individuals and possibly some species were not captured, especially in disturbed and early successional habitat where it is difficult to capture bats.

Based on mist-net captures and acoustic detections, bat populations in Martin and St. Lucie Counties are relatively higher (0.19 captures/hr, 9.5 bat-passes/hr) than bat populations in more developed counties to the south that include Broward, Dade, and Monroe where limited activity was documented during 11 survey nights (0 captures/hr, 0.08 bat-passes/hr; Hutchinson, 2003). The higher capture and detection rates recorded along the Treasure coast compared to counties located to the south may be attributed to more protected natural areas, less landscape fragmentation, numerous sources of freshwater, and a mosaic of forest habitat in various stages of succession.

All species of bats previously documented from the area were captured during the survey. An additional species, the eastern pipistrelle, was also documented during the survey (Hutchinson and Roberts, 2001). Evening bats appear to be habitat generalists in their foraging behavior. This is most likely a reflection of their selection of roost sites in both natural structures (e.g., snags, cavities, etc.) and anthropogenic structures (e.g., attics, eaves, etc.). Thus, the availability of roost sites may be one of the most important factors in bat species diversity as suggested by Humphrey (1975). The Brazilian free-tailed bat appears to be more roost- and possibly foraging-specialized than the other four species, being captured only in ruderal habitat which may be a function of its habit of selecting roost sites in anthropogenic structures and foraging in open areas. In the southeastern United States, the Brazilian free-tailed bat roosts almost exclusively in buildings and other structures (Barbour and Davis, 1969).

No species captured during the survey was listed as threatened or endangered. However, the status of two species, northern yellow and Brazilian free-tailed bats, is undetermined due to insufficient population data (Kern, 1992; Belwood, 1992). Based on this survey, the northern yellow bat appears to be uncommon along the Treasure Coast, but may be the most wide-ranging bat in southeast Florida (Robson, 1989). The Brazilian free-tailed bat appears to be locally abundant along the Treasure Coast, especially in residential areas. Surveys of pest control companies that exclude bats from human structures could provide some insight into the abundance and distributional patterns of this species throughout the state. Further research is needed on the selection of roost site of Brazilian free-tailed bats in peninsula Florida using radiotelemetry to determine the extent this species utilizes natural roosts in Florida. At Archbold Biological Station in Lake Placid, a maternity colony of Brazilian free-tailed and evening bats was observed roosting under bark and other crevices in a large slash pine snag (Hutchinson, unpubl. data).

Bats were captured and detected in all habitats and seasons indicating that bats utilize a variety of natural and ruderal habitats and are active throughout the year in southeast Florida. Capture rates of bats were highest in ruderal, mature scrub, and blackwater stream habitat, all of which contained large trees and canopy cover. High detection rates were recorded in ruderal and early successional habitat that contained low canopy cover and few but scattered trees, indicating that open area may be important foraging sites for bats. Seasonally, capture rates were higher in the spring and detection rates were highest in the winter; though limited winter surveys were conducted and all were conducted when the temperature was >21.0°C. Bat activity in southeast Florida appears to be limited once temperatures drop below 17.0°C (Hutchinson, 2001).

The lack of captures and detections of bats in pine flatwoods habitat is puzzling considering it is the most abundant habitat at JDSP. On three different occasions, bat detectors were utilized while driving <8 Km/hr through 12–16 Km of pine flatwoods habitat in JDSP to search for potential net sites, but no bats were detected. Based on results from acoustic and mist-net surveys, Robson (1989) stated that pine flatwoods was the second most-utilized habitat by bats in south Florida. Most pine flatwoods habitat in JDSP, SEPSP, and SAPSP contain numerous small slash pine (<20 cm) and few large slash pines (>45 cm). It is plausible that bats prefer mature, open, and frequently burned pine flatwoods for roosting and foraging similar to the habitat preferred by red-cockaded woodpeckers that contain a low density of large diameter slash pines (Hovis and Labisky, 1985). This habitat would provide an abundance of roost sites and open foraging area required by bats. An interesting study would be a comparison of bat activity in habitat that contains and does not contain red-cockaded woodpeckers. Recent studies have shown that cavity- and foliage-roosting bats prefer mature forest habitat with large living trees and large snags (Menzel et al., 1998, 1999; Lacki and Schwierjohann, 2001).

Mature tracts of upland forest are important consideration for bat conservation as larger trees and snags found in mature forest provide roost sites for cavity- and foliage- roosting species such as the evening, northern yellow, and Seminole bat. Roost lability has been associated principally with cavity- and foliage-roosting bats (Lewis, 1995), indicating these bats may require a large number of roost sites within the landscape. Surveys in urban areas have found that the most common bats were structural-roosting species accounting for over 80% of all bats, while limited numbers of snag- and tree-roosting bats were documented, an indication that natural roost sites were unavailable (Kurta and Teramino, 1992; Everette et al., 2001). Estrada and co-workers (1993) found that a negative relationship existed between bat species richness and distance to forest habitat.

As large-scale development continues along the Treasure Coast, the continual loss of natural habitat will undoubtedly not only affect bats but all species of wildlife. Bats use a wide array of the landscape for roosting (day, night, bachelor, and maternity roosts) and foraging (street lights, open fields, forest canopies, streams, lakes, etc.) that includes all natural communities documented in this survey. The ability of bats, due to their mobility and rapid flight, to utilize a large array of landscape features may buffer bats slightly more from landscape fragmentation than other species of wildlife because they utilize many habitats for roosting and foraging. Regardless, little information is known on the effects of habitat loss and fragmentation on bat populations. The cryptic nature of bats and their nocturnal nature make them less conspicuous than many other species of wildlife.

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LITERATURE CITED

ANTHONY, E. L. P. 1988. Age determination in bats. Pp. 47–58. *In*: Kunz, T. H. (ed.), Ecological and Behavioral Methods for the Study of Bats. Smith. Instit.Press, Washington, DC.

BALL, L. C. 2002. A strategy for describing and monitoring bat habitat. J. Wildl. Manage. 66:1148–1153.

BARBOUR, R. W. AND W. H. DAVIS. 1969. Bats of America. Univ. Kentucky Press, Lexington. 286 pp.

BELWOOD, J. J. 1981. Wagner's mastiff bat, *Eumops glaucinus floridanus*, (Molossidae) in southwestern Florida. J. Mammal. 62:411–413.

——— 1992. Brazilian free-tailed bat. Pp. 357–368. *In*: HUMPHREY, S. R. (ed.), Rare and Endangered Biota of Florida: Vol. 1. Mammals. Univ. Press Florida, Gainesville. 392 pp.

ESTRADA, A., R. COATES-ESTRADA, AND D. MERITT, JR. 1993. Bat species richness and abundance in tropical rain forests fragments and in agriculture habitats at Los Tuxtlas, Mexico. Ecography 16:309–318.

EVERETTE, A. L., T. J. O'SHEA, L. E. ELLISON, L. A. STONE, AND J. L. MCCANCE. 2001. Bat use of a high-plains urban wildlife refuge. Wild. Soc. Bull. 29:967–973.

HENRY, J. A., K. M. PORTIER, AND J. COYNE. 1994. The Climate and Weather of Florida. Pineapple Press, Inc. Sarasota, FL. 279 pp.

HOVIS, J. A. AND R. L. LABISKY. 1985. Vegetative associations of red-cockaded woodpecker colonies in Florida. Wild. Soc. Bull. 13:307–314.

HUMPHREY, S. R. 1975. Nursery roosts and community diversity of Nearctic bats. J. Mammal. 56:321–346.

HUTCHINSON, J. T. 2001. Observations on use of coastal scrub habitat by evening bats (*Nycticeius humeralis*) in Florida. Bat Res. News 42:44–46.

——— AND R. E. ROBERTS. 2001. Notes on the eastern pipistrelle in southeast Florida. Florida Field Nat. 29:54–55.

———. 2003. Cursory assessment of bat activity in state parks of coastal southeast Florida. Bat Res. News 44:50–53.

JENNINGS, W. L. 1958. The ecological distribution of bats in Florida. PhD Diss., Univ. of Florida, Gainesville, FL. 126 pp.

KERN, W. H., JR. 1992. Northern yellow bat. Pp. 349–356. *In*: HUMPHREY, S. R. (ed.), Rare and Endangered Biota of Florida: Volume 1. Mammals. Univ. Press Florida, Gainesville. 392 pp.

KUNZ, T. H. AND A. KURTA. 1988. Capture methods and holding devices. Pp. 1–29. *In*: Kunz, T. H. (ed.), Ecological and Behavioral Methods for the Study of Bats. Smith. Instit.Press, Washington, DC.

KURTA, A. AND J. A. TERAMINO. 1992. Bat community structure in an urban park. Ecography 15:257–261.

LACKI, M. J. AND J. H. SCHWIERJOHANN. 2001. Day-roost characteristics of northern bats in mixed mesophytic forest. J. Wildl. Manage. 65:482–488.

LEWIS, S. E. 1995. Roost fidelity of bats: a review. J. Mammal. 76:481–496.

MENZEL, M. A., T. C. CARTER, B. R. CHAPMAN, AND J. LAERM. 1998. Quantitative comparison of tree roosts used by red bats (*Lasiurus borealis*) and Seminole bats (*L. seminolus*). Can. J. Zool. 76:630–634.

———, D. M. KRISHON, T. C. CARTER, AND J. LAERM. 1999. Notes on tree roost characteristics of the northern yellow bat (*Lasiurus intermedius*), the Seminole bat (*L. seminolus*), the evening bat (*Nycticeius humeralis*), and the eastern pipistrelle (*Pipistrellus subflavus*). Florida Scient. 62:185–193.

MORGAN, G. S. 1991. Neotropical chiroptera from the Pliocene and Pleistocene of Florida. Bull. Am. Muse. Nat. Hist. 206:176–213.

MYERS, R. L. AND J. J. EWEL (eds.). 1990. Ecosystems of Florida. Univ. of Central Florida Press, Orlando, FL. 765 pp.

Robson, M. 1989. Status survey of the Florida mastiff bat. Final Performance Report. Florida Game and Freshwater Fish Commission, Bureau of Nongame Wildlife. Tallahassee, FL. 17 pp.

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COMPETITIVE INTERACTIONS BETWEEN THE SEA URCHIN *LYTECHINUS VARIEGATUS* AND EPIFAUNAL GASTROPOD GRAZERS IN A SUBTROPICAL SEAGRASS BED

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Abstract: *The sea urchin* Lytechinus variegatus *was introduced to a* Thalassia testudinum *bed in Biscayne Bay, Florida. Experimentally increased densities of urchins had no effect on seagrass biomass or shoot density. Seasonal effects on seagrass biomass and shoot density were highly significant, with higher values for both parameters in the fall. Total epifaunal gastropod abundances were significantly affected by urchin density, decreasing to almost zero in the high urchin density treatment. These results suggest that competition was occurring between the urchins and gastropods.*

Key Words: Biscayne Bay, Florida, competition, gastropods, herbivory, *Lytechinus variegatus*, seagrass, urchin

Seagrass beds support large and complex animal communities, both infaunal and epifaunal (Santos and Simon, 1974; Howard, 1987; Virnstein and Howard, 1987a; Gambi et al., 2003). Many of these fishes and invertebrates are herbivorous, feeding on live seagrass, macroalgae, or epiphytic algae (Randall, 1965; Heck and Orth, 1979; Lobel and Ogden, 1981; Orth and van Montfrans, 1984; Virnstein and Howard, 1987b; Klumpp et al., 1993; Cebrián and Duarte, 1998; Valentine and Heck, 1999; Kirsch et al., 2002). Consumption of live seagrass material, which is highly refractory and low in nutrition compared to many algae, is relatively rare, but does occur in some species of sea urchins and fishes (Moore et al., 1963; Randall, 1965; Camp et al., 1973; Lobel and Ogden, 1981; Klumpp et al., 1993; Montague et al., 1995; Cebrián and Duarte, 1998; Valentine and Heck, 1999; Kirsch et al., 2002). Consumption of epiphytes is more common, and gastropods are among the most common grazers of epiphytic algae (Orth and van Montfrans, 1984; Virnstein, 1987). Much work has been done on the effects of both urchin and gastropod grazers in seagrass beds, but to date no research has investigated the potential competition that may exist between these two groups of herbivores in this habitat.

In the tropical and subtropical western Atlantic, the primary invertebrate grazer of live seagrass biomass is the sea urchin *Lytechinus variegatus* (Greenway, 1976; Vadas et al., 1982; Zieman, 1982; Valentine and Heck, 1991; Maciá and Lirman,

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1999; Rose et al., 1999). Seagrass beds of this area are also home to an abundant and diverse epifaunal gastropod community (Jackson, 1972; Brook, 1978; Bauer, 1983; Virnstein and Howard, 1987a; Maciá, 2001). Gastropods inhabiting seagrass beds can undergo extreme interannual as well as seasonal fluctuations in abundance (Jackson, 1972; Hatfield, 1980; Nelson, 1997; Maciá, 2001). In some cases these fluctuations have been explained by seasonal recruitment patterns (Jackson, 1972; Maciá, 2001), differences in predation rates (Jackson, 1972), or temperature stress (Nelson, 1997), but in other cases the reasons are unclear. Because gastropod grazers are an important trophic link between the primary epiphytic producers of the seagrass bed and higher order consumers such as crustaceans and fishes, a better understanding of gastropod population dynamics is needed. Competition with other grazers, such as urchins, may have important effects on gastropod populations.

Despite the extensive research that has been conducted on grazing of seagrasses, no studies have investigated the effect of experimentally introduced urchin grazing on a seagrass community with no naturally occurring urchin population. The present study uses such an approach to determine the effect of novel grazing on a seagrass population and to investigate the possibility of an interaction between the two major groups of herbivorous seagrass invertebrates. My null hypotheses are: 1. abundance of the urchin (*Lytechinus variegatus*) has no effect on the abundance of epifaunal gastropod grazers in a seagrass bed; and 2. the introduction of urchins will have no effect on the biomass and shoot density of the seagrass *Thalassia testudinum*.

MATERIALS AND METHODS—I introduced urchins at a site (Sands Key) in Biscayne Bay, Florida (Fig. 1). The experimental site is shallow (approximately 1 m deep) and has a dense (>450 shoots m^{-2}) nearshore *T. testudinum* bed. *Lytechinus variegatus* did not occur at this site during monthly surveys from September 1997 to August 1998.

I used cages to manipulate sea urchin density. The circular cages used were the same as those described in Maciá (2000). Briefly, the cages were $2 m^2$ in area with sides 60 cm high and a square mesh size of 3.2 x 3.2 cm. Cage tops were made of flexible netting with a mesh size of 3.2 × 3.8 cm. Both cage controls (partial cages exactly the same as complete cages except for a 1 m opening along the circumference) and unmanipulated control plots (no cage structures) were established and monitored in the same way as the complete cages.

I repeated the experiment during two seasons: fall (October–November 1998) and spring (April–May 1999). I used the following treatments: 5 urchins m^{-2}, 20 urchins m^{-2}, partial cage controls (0 urchins m^{-2}), and unmanipulated controls (0 urchins m^{-2}). There were six replicates for each treatment. The experimental plots (cages and controls) were placed 1 m apart and randomly arranged into a rectangular grid (4 rows of 6 treatments each).

After the cages and control treatments were established, I took initial measurements of seagrass shoot density within each replicate. I counted all shoots in a haphazardly located 0.04 m^2 quadrat within each plot. The location of the quadrat was permanently marked with surveyor's flags so that it could be followed throughout the duration of the experiment. Introduced urchins (40–55 mm test diameter) were collected from a *T. testudinum* bed in northern Biscayne Bay (Stiltsville, Fig. 1). The appropriate number of urchins was added to the cages, and all tops were sewn on.

Each seasonal phase (spring and fall) of the experiment ran for 6 weeks. At the end of this time, I identified and counted all epifaunal gastropods within each plot. I counted all seagrass shoots within the permanently marked quadrats, and collected a seagrass biomass sample from the center of each cage/control plot with a corer. Cores were 15 cm in diameter and 25 cm deep. Seagrass samples were separated into live above- and below-ground biomass, rinsed in dilute (8.5%) phosphoric acid, and dried to constant weight at 70°C.

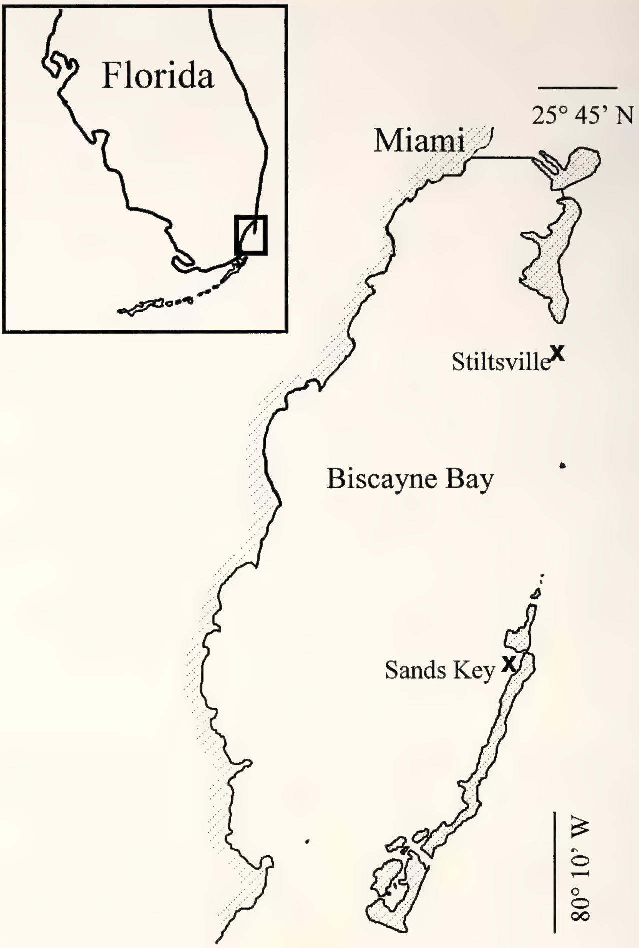

FIG. 1. Map of Biscayne Bay, Florida showing the Sands Key field site and Stiltsville collection site.

During each experiment, I checked the number of urchins in the cages approximately once a week. In the fall, few urchins were lost from the cages. Only one cage in the 20 m^{-2} treatment had a substantial loss of urchins (18 of the 40 remained in the cage throughout the 6 weeks). Thus, at the end of the fall experiment, actual urchin densities for the 5 m^{-2} and 20 m^{-2} treatments were 4.8 m^{-2} and 16.2 m^{-2}, respectively. During the spring experiment, however, large numbers of urchins escaped or died, especially from the 20 m^{-2} treatment. At the end of the experiment the average number of urchins was 3.8 m^{-2} and 11.8 m^{-2} for the 5 m^{-2} and 20 m^{-2} treatments, respectively. No escaped urchins were found in the seagrass surrounding the cages. Fouling on the cage sides and tops was minimal.

Statistical analyses—Assumptions of normality and homoscedasticity were tested with the Shapiro-Wilk test and the Bartlett test, respectively (alpha = 0.05), prior to all statistical analyses. When the assumptions of normality and homoscedasticity could not be met, the non-parametric Scheirer-Ray-Hare two-way extension of the Kruskal-Wallis test was used in lieu of a parametric two-way ANOVA (Sokal and Rohlf, 1995). Because shoot counts were conducted within the same permanently marked quadrat at

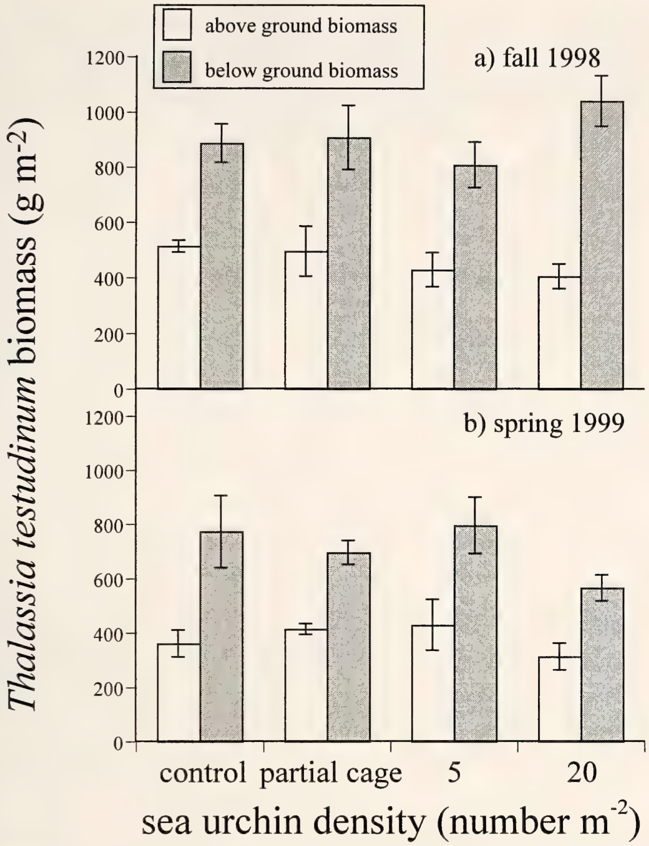

FIG. 2. Biomass of *Thalassia testudinum* (mean ± SE) in the urchin introduction experiment after six weeks. a) fall experiment (October–November 1998) and b) spring experiment (April–May 1999).

the beginning and end of the experiments, these data were compared with a repeated-measures ANOVA with season and urchin density as fixed factors. All the gastropods found were epifaunal grazers and therefore ecologically similar, thus they were grouped together for statistical analysis.

RESULTS—Urchin density had no significant effect on the biomass, either above- or below-ground, of *T. testudinum* (Fig. 2; Table 1). Even at the highest urchin density of 20 m^{-2}, seagrass biomass did not significantly decrease. There was an effect of season, however, with above- and below-ground biomass both significantly higher in the fall than in the spring (Table 1). Similarly, urchin density had no effect on shoot density of *T. testudinum* (Table 2). There was a highly significant effect of season on seagrass shoot density (Table 2), which was higher in the fall (Fig. 3). No cage effects (i.e., significant differences between unmanipulated controls and cage controls) were detected in any of these analyses.

The species of gastropods occurring at the site included: *Lithopoma americana*, *Modulus modulus*, *Turbo castanea*, *Columbella rusticoides*, and *Cerithium* sp. Comparison of gastropod density in cage controls and unmanipulated control plots

TABLE 1. Analysis of variance for *Thalassia testudinum* biomass in the urchin introduction experiment with season and urchin density as factors. Assumptions of both normality and homoscedasticity were met only for below-ground biomass. Above-ground biomass was analyzed with the non-parametric Scheirer-Ray-Hare 2-way extension of the Kruskal-Wallis ANOVA.

Above-ground biomass				
Source	df	SS	H	p
urchin density	3	89.50	0.15	0.984
season	1	1728.00	8.81	0.003
urchin * season	3	1035.17	1.76	0.624
error	40	6359.33		

Below-ground biomass				
Source	df	SS	F	p
urchin density	3	2.06	0.04	0.988
season	1	162.69	10.09	0.003
urchin * season	3	116.18	2.40	0.082
error	40	16.12		

for both seasons indicated that there were no cage effects (Scheirer-Ray-Hare non-parametric two-way ANOVA: $p = 0.98$), therefore the cage controls were excluded from further analyses. Sea urchin density significantly affected the total number of gastropods (Table 3). In the high urchin density cages, gastropod density declined nearly to zero (Fig. 4). There was no effect of season on gastropod abundance (Table 3).

DISCUSSION—This study tested the effects of sea urchin grazing on a seagrass population and its epifaunal gastropod community. Regardless of season, urchin grazing had no effect on seagrass biomass or shoot density, even at the high density of 20 urchins m^{-2}. Seasonal effects independent of urchin grazing, however, were highly significant for the seagrass population. Both biomass and shoot density of *T. testudinum* were significantly higher in the fall (October–November), just after the peak summer growing season, than in the spring (April–May). This seasonal pattern agrees with other studies of *T. testudinum*, which show maximum biomass in the warmer months (Zieman, 1975; Dawes et al., 1985; vanTussenbroek, 1995; Lee and Dunton, 1996; Gacia, 1999; Irlandi et al., 2002). My results indicate that the abundance of turtlegrass at the study site is influenced primarily by seasonal effects rather than by grazing.

The abundance of urchins did significantly affect the epifaunal gastropod grazers (*Lithopoma americana*, *Modulus modulus*, *Turbo castanea*, *Columbella rusticoides*, and *Cerithium* sp.). At the highest urchin density (20 m^{-2}), gastropod abundance dropped nearly to zero, in both spring and fall. These gastropods feed primarily on epiphytes growing on the seagrass blades (Emerson and Jacobson, 1976; Orth and van Montfrans, 1984; Virnstein, 1987). Urchins, including *Lytechinus variegatus*, will consume epiphytic algae, but only while also consuming

TABLE 2. Results of repeated measure ANOVA analysis on *Thalassia testudinum* shoot counts in permanently marked quadrats at the beginning and end of the urchin introduction experiment. "Time" refers to sampling time at the beginning and end of the experiment (initial and final).

Source	SS	df	MS	F	P
Between Subjects					
urchin density	0.689	3	0.230	1.427	0.249
season	5.740	1	5.740	35.682	0.000
urchin * season	0.231	3	0.077	0.478	0.699
error	6.435	40	0.161		
Within Subjects					
time	0.000	1	0.000	0.000	0.998
time * urchin density	0.260	3	0.087	2.095	0.116
time * season	0.068	1	0.068	1.645	0.207
time * urchin * season	0.139	3	0.046	1.123	0.351
error	1.654	40	0.041		

the seagrass blades to which they are attached (Greenway, 1976; Keller, 1983; Beddingfield and McClintock, 1999; Tertschnig, 1989; pers. obs.). Thus it does not appear that the urchins were outcompeting the gastropods by consuming all available food before the gastropods had an opportunity to feed (i.e., exploitative competition). No empty shells were found in or around the cages, indicating that the gastropods simply migrated out of the cages in response to increased sea urchin numbers.

Although exploitative competition between urchins and gastropods does not seem likely, the apparent migration response on the part of the gastropods nevertheless suggests some type of competitive interaction. This interaction could be in the form of interference competition, in which the urchins somehow prevent the gastropods from reaching their food source. Interference competition has been reported for the urchin *Strongylocentrotus purpuratus*, which can physically prevent the gastropod *Astraea undosa* from reaching the macroalgae that both species consume (Aguilar Rosas et al., 1990). Qualitative observations of tropical seagrass beds in the Philippines found that echinoderms (including echinoids, holothurians, and asteroids) were most numerous in areas with low levels of gastropod grazing (Klumpp et al., 1992), again suggesting the possibility of a competitive interaction between these two groups of grazers (although no competitive mechanism was postulated). Considering the high density of seagrass in the present study, however, the experimental densities of urchins do not seem high enough to be responsible for such a strong response in the gastropods. Thus, the exact mechanism of competition between the two groups of grazers is unclear.

In contrast with the present findings, in temperate rocky habitats gastropod and urchin densities are often positively correlated, and removal of urchins leads to a decrease in the abundance, shell size, and gonad size of these co-occurring gastropods (Ayling, 1981; Andrew and Choat, 1982; Fletcher, 1987). Thus, the effect of urchins on gastropod grazers will depend on the habitat and species in question. In temperate rocky areas, urchin grazing reduces the abundance of foliose

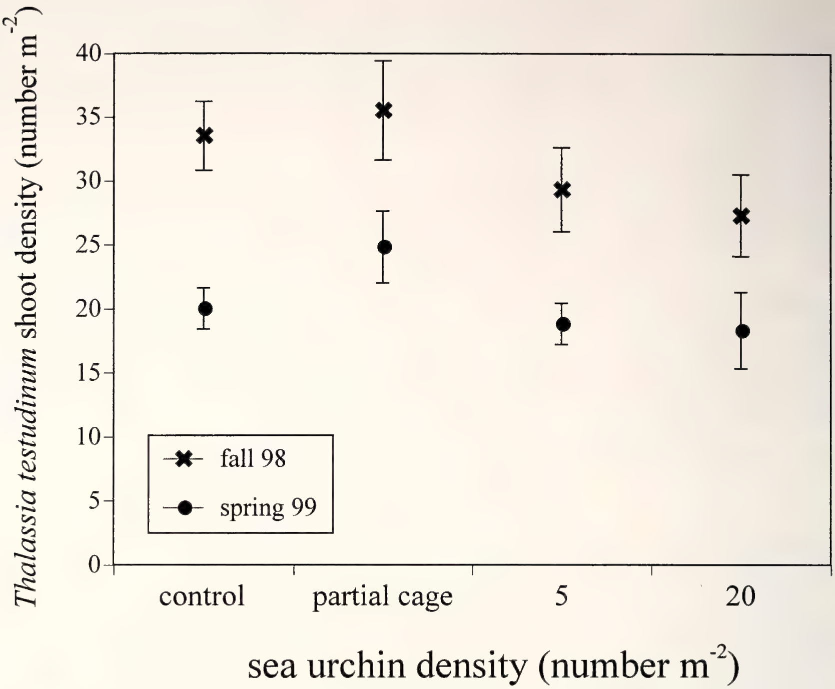

FIG. 3. Shoot density of *Thalassia testudinum* (mean ± SE) after six weeks of experimentally introduced urchin grazing.

algae, making space available for the encrusting coralline algae preferred by gastropods (Fletcher, 1987). In contrast, at my study site—a subtropical seagrass bed—the presence of urchins caused a decrease in gastropod populations. This difference is largely a result of the food source consumed by the species in each habitat: in rocky areas, gastropods and urchins are exploiting different food sources and therefore do not compete, while in the seagrass beds both herbivores are feeding on essentially the same food source (seagrasses or the epiphytes attached to them).

Egg predation is another potential mechanism whereby urchins could cause a decrease in gastropod abundance. *Lytechinus variegatus* has been known to preferentially consume gastropod egg masses (Beddingfield and McClintock, 1999). However, it is unlikely that this type of predation would have an effect on gastropod populations over the short time period (6 weeks) of my experiment.

TABLE 3. Analysis of variance for gastropod density with season and sea urchin density as factors. The data were analyzed with the non-parametric Scheirer-Ray-Hare 2-way extension of the Kruskal-Wallis ANOVA.

Source	df	SS	H	p
urchin density	2	2704.54	9.93	0.007
season	1	47.84	0.43	0.512
urchin * season	2	170.93	0.77	0.680
error	30	5975.42		

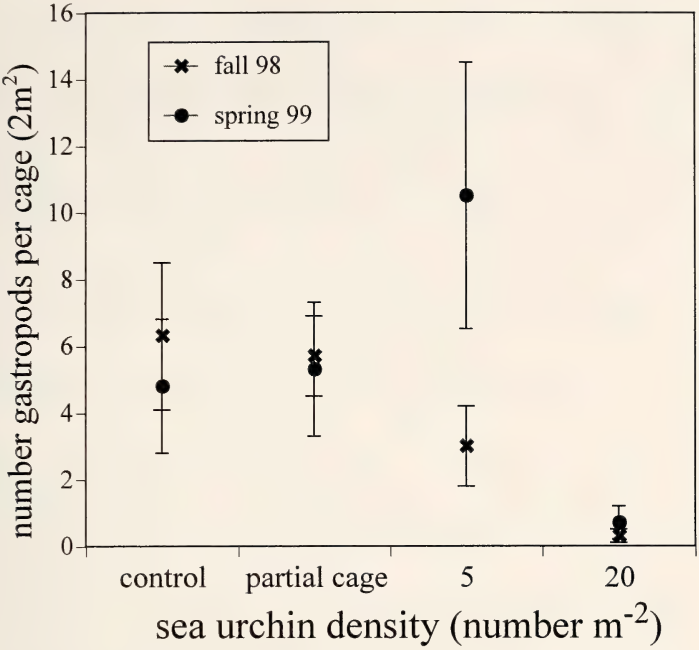

FIG. 4. Abundance of epifaunal gastropods after six weeks of experimentally introduced urchin grazing.

One potential problem with interpretation of my results is that many sea urchins escaped during the spring phase, leaving the cages with experimental densities below those originally planned (3.8 m^{-2} and 11.8 m^{-2} for the 5 m^{-2} and 20 m^{-2} treatments, respectively). Sea urchin densities of 3.8 and 11.8 m^{-2}, however, are still relatively high considering that the site normally has no sea urchins at all. Furthermore, treatment densities during the fall phase (4.8 m^{-2} and 16.2 urchins m^{-2}) remained close to the target values. I believe the experiment was effective in testing the effect of increased grazing pressure on the seagrass population and its gastropod community. Although the mechanism of interaction is uncertain, my results show that high abundance of sea urchins can have a significant negative impact on the abundance of epifaunal gastropod grazers.

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LITERATURE CITED

AGUILAR ROSAS, R., G. TORRES MOYE, AND A. ALMANZA HEREDIA. 1990. Análisis cualitativo de la dieta macroalgal del caracol *Astraea undosa* Wood 1828, en Puntta Banda, Baja California, México. Cien. Mar. 16:111–120.

AYLING, A. M. 1981. The role of biological disturbance in temperate subtidal encrusting communities. Ecology 62:830–847.

ANDREW, N. L. AND J. H. CHOAT. 1982. The influence of predation and conspecific adults on the abundance of juvenile *Evechinus chloroticus* (Echinoidea: Echinometridae). Oecologia 54:80–87.

BAUER, R. T. 1983. Mobile invertebrate epifauna from seagrass beds on the north coast of Puerto Rico: Diel variation in species composition and abundance. Proc. Assoc. Isl. Mar. Lab. Caribb. 17:13.

BEDDINGFIELD, S. D. AND J. B. MCCLINTOCK. 1999. Food resource utilization in the sea urchin *Lytechinus variegatus* in contrasting shallow-water microhabitats of Saint Joseph Bay, Florida. Gulf Mexico Sci. 17:27–34.

BROOK, I. M. 1978. Comparative macrofaunal abundance in turtlegrass (*Thalassia testudinum*) communities in South Florida characterized by high blade density. Bull. Mar. Sci. 28:212–217.

CAMP, D. K., S. P. COBB, AND J. F. VAN BREEDVELD. 1973. Overgrazing of seagrasses by a regular urchin, *Lytechinus variegatus*. Bioscience 23:37–38.

CEBRIÁN, J. AND C. M. DUARTE. 1998. Patterns in leaf herbivory on seagrasses. Aquat. Bot. 60:67–82.

DAWES, C. J., M. O. HALL, AND R. K. REICHERT. 1985. Seasonal biomass and energy content in seagrass communities on the west coast of Florida. J. Coastal Research. 1:255–262.

EMERSON, W. K. AND M. K. JACOBSON. 1976. The American Museum of Natural History Guide to Shells, Alfred A. Knopf, New York, NY.

FLETCHER, W. J. 1987. Interactions among subtidal Australian sea urchins, gastropods, and algae: effects of experimental removals. Ecol. Monogr. 57:89–109.

GACIA, E. 1999. Leaf dynamics and shoot production of the seagrass *Thalassia testudinum* in the Indian River Lagoon (Florida). Bot. Mar. 42:97–102.

GAMBI, M. C., B. I. VAN TUSSENBROEK, AND A. BREARLY. 2003. Mesofaunal borers in seagrasses: world-wide occurrence and a new record of boring polychaetes in the Mexican Caribbean. Aquat. Bot. 76:65–77.

GREENWAY, M. 1976. The grazing of *Thalassia testudinum* in Kingston Harbour, Jamaica. Aquat. Bot. 2:117–126.

HATFIELD, E. B. 1980. Natural history and population fluctuation of the gastropod *Anachis avara* (Say) in a tropical seagrass habitat, Miami, Florida. Bull. Mar. Sci. 30:604–612.

HECK, K. L. AND R. J. ORTH. 1979. Seagrass habitats: the roles of habitat complexity, competition and predation in structuring associated fish and motile macroinvertebrate assemblages. Pp. 449–464. *In*: KENNEDY, V. S. (ed.), Estuarine Perspectives. Academic Press, New York, NY.

HOWARD, R. K. 1987. Diel variation in the abundance of epifauna associated with seagrasses of the Indian River, Florida, USA. Mar. Biol. 96:137–142.

IRLANDI, E., B. ORLANDO, S. MACIÁ, P. BIBER, T. JONES, L. KAUFMAN, D. LIRMAN, AND E. PATTERSON. 2002. The influence of freshwater runoff on biomass, morphometrics, and production of *Thalassia testudinum*. Aquat. Bot. 72(1): 67–78.

JACKSON, J. B. 1972. The ecology of the molluscs of *Thalassia* communities, Jamaica, West Indies: II. Molluscan population variability along an environmental stress gradient. Mar. Biol. 14:304–337.

KELLER, B. D. 1983. Coexistence of sea urchins in seagrass meadows: an experimental analysis of competition and predation. Ecology 64:1581–1598.

KIRSCH, K. D., J. F. VALENTINE, AND K. L. HECK. 2002. Parrotfish grazing on turtlegrass *Thalassia testudinum*: evidence for the importance of seagrass consumption in food web dynamics of the Florida Keys National Marine Sanctuary. Mar. Ecol. Prog. Ser. 227:71–85.

KLUMPP, D. E., J. T. SALITA-ESPINOSA, AND M. D. FORTES. 1992. The role of epiphytic periphyton and macroinvertebrate grazers in the trophic flux of a tropical seagrass community. Aquat. Bot. 43:327–349.

———. 1993. Feeding ecology and trophic role of sea urchins in a tropical seagrass community. Aquat. Bot. 45:205–229.

LEE, K. AND K. H. DUNTON. 1996. Production and carbon reserve dynamics of the seagrass *Thalassia testudinum* in Corpus Christi Bay, Texas, USA. Mar. Ecol. Prog. Ser. 143:201–210.

LOBEL, P. S. AND J. C. OGDEN. 1981. Foraging by the herbivorous parrotfish *Sparisoma radians*. Mar. Biol. 64:173–183.

MACIÁ, S. 2000. The effects of sea urchin grazing and drift algal blooms on a subtropical seagrass bed community. J. Exp. Mar. Biol. Ecol. 246:53–67.

———. 2001. Population dynamics, growth rate, and habitat preference of the turbinid gastropod *Lithopoma americana* in Biscayne Bay, Florida. Nautilus 115:62–67.

——— AND D. LIRMAN. 1999. Destruction of Florida Bay seagrasses by a grazing front of sea urchins. Bull. Mar. Sci. 65:593–601.

MONTAGUE, J. R., J. L. CARBALLO, L. M. VALDES, AND M. CHACKEN. 1995. Analyses of decay and parrot fish grazing along attached blades of turtle grass (*Thalassia testudinum*) from two sites in Biscayne Bay. Florida Scient. 58:206–215.

MOORE, H. B., T. JUTARE, J. C. BAUER, AND J. A. JONES. 1963. The biology of *Lytechinus variegatus*. Bull. Mar. Sci. 13:23–53.

NELSON, T. A. 1997. Interannual variation in a subtidal eelgrass community. Aquat. Bot. 56:245–252.

ORTH, R. J. AND J. VAN MONTFRANS. 1984. Epiphyte-seagrass relationships with an emphasis on the role of micrograzing: a review. Aquat. Bot. 18:43–69.

RANDALL, J. E. 1965. Grazing effect on sea grasses by herbivorous reef fishes in the West Indies. Ecology 46:255–260.

ROSE, C. D., W. C. SHARP, W. J. KENWORTHY, J. H. HUNT, W. G. LYONS, E. J. PRAGER, J. V. VALENTINE, M. O. HALL, P. E. WHITFIELD, AND J. W. FOURQUREAN. 1999. Overgrazing of a large seagrass bed by the sea urchin *Lytechinus variegatus* in outer Florida Bay. Mar. Ecol. Prog. Ser. 190:211–222.

SANTOS, S. L. AND J. L. SIMON. 1974. Distribution and abundance of the polychaetous annelids in a south Florida estuary. Bull. Mar. Sci. 24:669–689.

SOKAL, R. R. AND F. J. ROHLF. 1995. Biometry, W. H. Freeman and Co., New York, NY.

TERTSCHNIG, W. P. 1989. Diel activity patterns and foraging dynamics of the sea urchin *Tripneustes ventricosus* in a tropical seagrass community and a reef environment (Virgin Islands). Mar. Ecol. 10:3–21.

VADAS, R. L., T. FENCHEL, AND J. C. OGDEN. 1982. Ecological studies on the sea urchin, *Lytechinus variegatus*, and the algal-seagrass communities of the Miskito Cays, Nicaragua. Aquat. Bot. 14:109–125.

VALENTINE, J. F. AND K. L. HECK. 1991. The role of sea urchin grazing in regulating subtropical seagrass meadows: evidence from field manipulations in the northern Gulf of Mexico. J. Exp. Mar. Biol. Ecol. 154:215–230.

———. 1999. Seagrass herbivory: evidence for the continued grazing of marine grasses. Mar. Ecol. Prog. Ser. 176:291–302.

VAN TUSSENBROEK, B. I. 1995. *Thalassia testudinum* leaf dynamics in a Mexican Caribbean coral reef lagoon. Mar. Biol. 122:33–40.

VIRNSTEIN, R. W. 1987. Seagrass-associated invertebrate communities of the southeastern U. S. A.: a review. Fl. Mar. Res. Publ. 42:89–115.

——— AND R. K. HOWARD. 1987a. Motile epifauna of marine macrophytes in the Indian River Lagoon, Florida. I. Comparisons among three species of seagrasses from adjacent beds. Bull. Mar. Sci. 41:1–12.

———. 1987b. Motile epifauna of marine macrophytes in the Indian River Lagoon, Florida. II. Comparisons between drift algae and three species of seagrasses. Bull. Mar. Sci. 41:13–26.

ZIEMAN, J. C. 1975. Seasonal variation of turtle grass, *Thalassia testudinum* König, with reference to temperature and salinity. Aquat. Bot. 1:107–123.

———. 1982. The ecology of the seagrasses of South Florida: a community profile, U.S. Fish Wildlife Service Biological Service Program, Report No. FWS/OBS–82/25. Washington, D.C.

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A NEW EXOTIC SPECIES IN FLORIDA, THE BLOODSUCKER LIZARD, *CALOTES VERSICOLOR* (DAUDIN 1802) (SAURIA: AGAMIDAE)

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Abstract: *We document an established population of an Asian agamid lizard commonly called the bloodsucker (*Calotes versicolor*) in St. Lucie County, Florida. This population was apparently introduced in 1978, and approximately 100 specimens have been captured by two commercial collectors in the past 10 yr. All specimens were captured from nocturnal roosts 1–9 m high in weeds, vines, bushes, and trees. We deposited voucher specimens consisting of 10 juveniles and an adult female with 19 oviductal eggs captured in August 2003. The known population boundaries extend ca. 10 km east-west and north-south, and they encompass citrus groves, Brazilian pepper (*Schinus terebinthifolius*) thickets, mesic flatwoods, and canals. We do not know what ecological impacts this species might have on native wildlife species, although it has been documented feeding on small vertebrates in Asia.*

Key Words: *Calotes versicolor*, Agamidae, Bloodsucker, Oriental Garden Lizard, Distribution, Exotics, Florida, St. Lucie County

Many non-native lizard species of tropical origin have become established in southern Florida, and Florida now has more nonindigenous amphibian and reptile species than any other state (Butterfield et al., 1997). In this paper, we report the first established population of *Calotes versicolor* (Daudin 1802), an Asian agamid lizard, in Florida. Butterfield and co-workers (1997) included *Calotes versicolor* in their list of established exotic herpetofaunal species in Florida based upon a personal communication from R. D. Bartlett. However, the purported *C. versicolor* population was misidentified and was actually *C. mystaceus* (Bartlett, 2003), which occurs in two small areas in the vicinity of pet dealerships in Glades and Okeechobee counties (Bartlett and Bartlett, 1999).

Of the 21 recognized species of *Calotes* (Vindum et al., 2003), *C. versicolor* is the most widespread, ranging from southeastern Iran and Afghanistan east to Indo-China and as far south as Sri Lanka, Sumatra, and northern Peninsular Malaysia (Boulenger, 1912; Smith, 1935; Zhao and Adler, 1993; Cox et al., 1998). *Calotes versicolor* is distinguished from its congeners by having lateral body scales pointing backwards and upwards, no oblique fold or pit anterior to the shoulder, two separated spines above the tympanum, and 35–52 scales around the body (Smith, 1935). *Calotes versicolor* exhibits obvious geographic variation in coloration, scalation, and size across its wide range, but only two subspecies have been described (Auffenberg and Rehman, 1993, 1995); our specimens were of the nominate subspecies.

Males may measure 140 mm snout-vent length (SVL) and have a slender tail 295 mm long (Radder et al., 2001). Adults have large heads, massive shoulders, expandable dewlaps, and laterally flattened bodies with a crest extending from the neck almost to the tail. The ground color is typically dull brown, gray, or olive with irregular dark brown spots or bars, but breeding males have pale yellow bodies with a large black patch on each side of the throat. Breeding males also develop bright orange or crimson areas around the head and shoulders, but this brilliant coloration can change rapidly depending upon mood or environmental conditions (Bhatti and Bhatti, 1986; Cox et al., 1998). This red coloration accounts for these lizards commonly being called bloodsuckers. Other names for this arboreal species are the oriental or tropical garden lizard, Indian tree lizard, crested tree lizard, and changeable lizard.

METHODS—Most of our information was obtained from two commercial collectors, G. Ward and M. Flowers, who notified us of this population and provided the first voucher specimen. On 19 August 2003, we accompanied the two collectors on a nighttime visit to the locality (27°16′43.2″N, 80°32′18.0″W) ca. 16 km west of Port St. Lucie, St. Lucie County, Florida. The two collectors have captured specimens here for the past 10 yr by shining lights in weeds, shrubs, and trees. Voucher specimens were deposited in the Florida Museum of Natural History (FLMNH), University of Florida (UF collection). *Calotes versicolor* were measured to the nearest mm (± S.D.) using a ruler, and weighed to the nearest 0.1 g using Pesola spring scales.

RESULTS—A gravid adult female measuring 108 mm SVL and 378 mm total length (TL) was collected at 2230 hr on 15 August 2003 by G. Ward (UF 137448). This female was set up in an enclosure but died on 31 August 2003. When dissected, she was found to contain 19 oviductal eggs measuring 15.2 ± 0.73 mm (range = 14.6–15.9 mm) long and 8.0 ± 0.32 mm (range = 7.4–8.5 mm) wide. During our visit on 19 August 2003, 10 juveniles (UF 137449–58) were collected from 2200 to 2215 hr while sleeping on the inflorescences of goldenrod (*Solidago* sp.) along a 10-m stretch on the east bank of a canal, ca. 30 m north of where the gravid female was collected four days earlier. This canal runs along the west side of a small, north-south dirt road 0.8 km west of Carlton Road. The juveniles were found in a 2-m-wide swath of grasses and weeds above a steep bank leading down to a canal. On the east side of the dirt road bordering the canal is a Brazilian pepper (*Schinus terebinthifolius*) thicket, and citrus groves line the west side of the canal. The juveniles measured 31.0 ± 0.94 mm SVL (range = 29–32 mm) and 96.7 ± 3.71 mm TL (range = 89–101 mm), and each one had a mass of 0.6 g.

DISCUSSION—Approximately 100 adult *Calotes versicolor* have been collected from this population in the past 10 yr by two commercial collectors, but our visit produced the first juveniles (Flowers, 2003; Ward, 2003). Reproduction in this population has undoubtedly occurred previously, but neonates and juveniles were not detected. Adults have been collected from May through October while roosting singly at night 1–9 m above the ground in shrubs, vines, or trees such as Brazilian pepper, cabbage palm (*Sabal palmetto*), or slash pine (*Pinus elliottii*) (Flowers, 2003; Ward, 2003). Few *C. versicolor* are collected on a typical night; collectors receive $2–5 per adult lizard (Flowers, 2003).

Several *Calotes* escaped in 1978 from a reptile dealer located near the southwestern end of Glades Cutoff Road (Weinberg, 2003). The escapees came from a shipment from Pakistan, and the reptile dealer told us that the established population consisted of *C. emma*. However, the species present is actually *C. versicolor*, the only *Calotes* that occurs in Pakistan (Auffenberg and Rehman, 1993). The approximate boundaries of the population have been determined by extensive collection by two commercial collectors (Flowers, 2003; Ward, 2003). The southeastern boundary of the population is apparently Glades Cutoff Road, indicating that the population has expanded mostly northwards. The known northern population boundary is 27°20′15″N, the southern boundary is 27°15′00″N, the western boundary is 80°35′00″W, and the eastern boundary is 80°27′30″W. The known boundaries of this population extend for a maximum of 11.7 km east-west and 9.3 km north-south, and the area encompassed mostly consists of citrus groves dissected by canals and interspersed with thickets of Brazilian pepper and patches of mesic flatwoods.

The 10 juveniles (mean SVL of 31 mm) collected on 19 August 2003 may not have been neonates, because neonates in India typically measure 25–28 mm SVL (Radder et al., 2001). However, all were collected within 10 m of each other, suggesting that they may not have dispersed far since hatching from a single clutch; no other *Calotes versicolor* were observed while searching approximately 100 m of adjacent canal banks. The gravid female collected measured 108 mm SVL, which is larger than females from most populations (Auffenberg and Rehman, 1993; Ji et al., 2002) but falls within the range of adult females from peninsular India (86–136 mm SVL) (Radder et al., 2001). Clutch size is positively correlated with SVL (Shanbhag et al., 2000; Ji et al., 2002), and the clutch of 19 eggs from our female corresponds with the appropriate SVL for females from India, where the maximum clutch size was 33 eggs (Shanbhag et al., 2000; Shanbhag, 2003). In India, *C. versicolor* oviposits multiple clutches May–October (Shanbhag and Prasad, 1993), and clutch size is larger in early and mid-breeders than in late breeders of the same size (Shanbhag et al., 2000). The eggs in our female were slightly larger than those from late-breeding females in India (Shanbhag et al., 2000). Interestingly, gravid females have the ability to retain oviductal eggs for six months or longer under unfavorable conditions by lowering their body temperature by 3–5°C and arresting developing embryos at stage 34 (Shanbhag, 2003; Shanbhag et al., 2003).

In its native range, *Calotes versicolor* is commonly observed in parks, gardens, agricultural areas, waste land, and open forests (Cox et al., 1998). They typically live among leafy undergrowth and grass in open habitats, but males often display from fences and other conspicuous perches (Cox et al., 1998). Juveniles forage and bask mostly at ground level, whereas subadults and adults spend much of their time on tree trunks, often in a head-down posture surveying for prey (Diong et al., 1994).

Calotes versicolor is a very adaptable, prolific species that is commonly found in human-altered environments and can survive even in urban areas in Asia (Erdelen, 1988). In its native range, *C. versicolor* preys primarily on arthropods, but adults prey occasionally on their own young as well as small birds and nestlings, frogs, geckos, and small snakes (Rao, 1975; Dhindsa and Toor, 1983; Sharma, 1991a, 1991b, 1999; Diong, 1994). *Calotes versicolor* was introduced into Singapore in the 1980s, where it rapidly established populations (Chou, 1994) and partially displaced populations of a native agamid, the green crested lizard (*Bronchocela cristatella*) (Diong et al., 1994). We do not know what effect *C. versicolor* will have on populations of sympatric native lizard species in Florida, such as the green anole (*Anolis carolinensis*), ground skink (*Scincella lateralis*), and southeastern five-lined skink (*Eumeces inexpectatus*). Populations of the non-native brown anole (*Anolis sagrei*) and knight anole (*A. equestris*) are already common in the area, and non-native brown basilisks (*Basiliscus vittatus*) and green iguanas (*Iguana iguana*) are occasionally observed in adjacent areas (Flowers, 2003; Ward, 2003). *Calotes versicolor* is yet another exotic species that may compete with or prey upon Florida's native species.

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LITERATURE CITED

AUFFENBERG, W. AND H. REHMAN. 1993. Studies on Pakistan reptiles. Pt. 3. *Calotes versicolor*. Asiatic Herpetol. Res. 5:14–30.

——— AND H. REHMAN. 1995. *Calotes versicolor nigrigularis* Auffenberg and Rehman 1933 a junior primary homonym. Asiatic Herpetol. Res. 6:27.

BARTLETT, R. D. 2003. Gainesville, FL. Pers. Comm.

——— AND P. P. BARTLETT. 1999. A Field Guide to Florida Reptiles and Amphibians. Gulf Publishing, Houston, TX. 280 pp.

BHATTI, U. S. AND S. K. BHATTI. 1986. Mating behavior of garden lizard *Calotes versicolor*. Environ. Ecol. 4:183–184.

BOULENGER, G. A. 1912. A Vertebrate Fauna of the Malay Peninsula from the Isthmus of Kra to Singapore, Including the Adjacent Islands. Reptilia and Batrachia. Taylor and Francis, London. 294 pp.

BUTTERFIELD, B. P., W. E. MESHAKA, Jr., AND C. GUYER. 1997. Nonindigenous amphibians and reptiles. Pp. 123–138. *In*: SIMBERLOFF, D., D. C. SCHMITZ, AND T. C. BROWN (eds.). Strangers in Paradise: Impact and Management of Nonindigenous Species in Florida. Island Press, Washington, D.C.

CHOU, L. M. 1994. *Calotes versicolor* (Indian tree lizard). Herpetol. Rev. 25:75–76.

COX, M. J., P. P. VAN DIJK, J. NABHITABHATA, AND K. THIRAKHUPT. 1998. A Photographic Guide to Snakes and Other Reptiles of Peninsular Malaysia, Singapore and Thailand. Ralph Curtis Books, Sanibel, FL. 144 pp.

DHINDSA, M. S. AND H. S. TOOR. 1983. Reciprocal predation between weaver birds and a lizard species. J. Bombay Nat. Hist. Soc. 80:221–222.

DIONG, C. H. 1994. *Calotes versicolor* (oriental garden lizard). Cannibalism and diet. Herpetol. Rev. 25:25–26.

———, L. M. CHOU, AND K. K. P. LIM. 1994. *Calotes versicolor*: The changeable lizard. Nature Malaysiana 19:46–54.

ERDELEN, W. 1988. Population dynamics and dispersal in three species of agamid lizards in Sri Lanka: *Calotes calotes, C. versicolor* and *C. nigrilabis*. J. Herpetol. 22:42–52.

FLOWERS, M. A. 2003. MG Reptiles, Vero Beach, FL. Pers. Comm.

JI, X., Q.-B. QIU, AND C. H. DIONG. 2002. Sexual dimorphism and female reproductive characteristics in the oriental garden lizard, *Calotes versicolor*, from Hainan, southern China. J. Herpetol. 36:1–8.

RADDER, R. S., B. A. SHANBHAG, AND S. K. SAIDAPUR. 2001. Ontogeny of sexual dimorphism in the tropical garden lizard, *Calotes versicolor* (Daud.). J. Herpetol. 35:156–160.

RAO, S. M. V. 1975. Studies on the food and feeding behaviour of the agamid garden lizards *Calotes versicolor*. Brit. J. Herpetol. 5:467–470.

SHANBHAG, B. A. 2003. Reproductive strategies in the lizard, *Calotes versicolor*. Current Sci. 84:646–652.

——— AND B. S. K. PRASAD. 1993. Follicular dynamics and germinal bed activity during annual ovarian cycle in the lizard, *Calotes versicolor* (Daud.). J. Morphol. 215:1–7.

———, R. S. RADDER, AND S. K. SAIDAPUR. 2000. Maternal size determines clutch mass, whereas breeding timing influences clutch and egg sizes in the tropical lizard, *Calotes versicolor* (Agamidae). Copeia 2000:1062–1067.

———, S. K. SAIDAPUR, AND R. S. RADDER. 2003. Lowering body temperature induces embryonic diapause during prolonged egg retention in the lizard, *Calotes versicolor*. Naturwiss. 90:33–35.

SHARMA, S. K. 1991a. Cannibalism by common garden lizard *Calotes versicolor*. J. Bombay Nat. Hist. Soc. 88:290–291.

———. 1991b. Common garden lizard *Calotes versicolor* preying on Brook's gecko *Hemidactylus brooki*. J. Bombay Nat. Hist. Soc. 88:459.

———. 1999. *Calotes versicolor* feeding on *Lycodon aulicus*. J. Bombay Nat. Hist. Soc. 96:146–147.

SMITH, M. A. 1935. The Fauna of British India, Including Ceylon and Burma. Reptilia and Amphibia. Vol. 2. Sauria. Taylor and Francis, London. 440 pp.

VINDUM, J. V., H. WIN, T. THIN, K. S. LWIN, A. K. SHEIN, AND H. TUN. 2003. A new *Calotes* (Squamata: Agamidae) from the Indo-Burman Range of western Myanmar (Burma). Proc. Calif. Acad. Sci. 54:1–16.

WARD, G. J. 2003. MG Reptiles, Vero Beach, FL. Pers. Comm.

WEINBERG, A. 2003. Allapattah Flats Turtle Preserve, Port St. Lucie, FL. Pers. Comm.

ZHAO, E.-M. AND K. ADLER. 1993. Herpetology of China. Society for the Study of Amphibians and Reptiles, Oxford, OH. 522 pp.

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A SIMPLE ONE-STEP PURIFICATION OF RbsD OF THE D-RIBOSE HIGH-AFFINITY TRANSPORT SYSTEM OF *ESCHERICHIA COLI*

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Abstract: *The D-ribose high-affinity transport operon (RbsDACBKR) of* Escherichia coli *encodes the proteins necessary for high-affinity transport and utilization of D-ribose. RbsD encodes a 15 kDa protein and is the first gene of the operon. The gene has been cloned into a T7-based expression system and expressed in E. coli. The gene product has been purified in a single step using hydrophobic interaction chromatography. This simple, fast and efficient purification protocol should facilitate detailed characterization of the protein.*

Key Words: RbsD, protein purification, D-ribose transport

Protein purification is often the rate-limiting step in the analysis of the structure-function relationship of a protein. The ability to overexpress a gene product has greatly facilitated protein purification in that a specific protein can now potentially be expressed to become the most abundant protein in a cell. The bacteriophage T7 RNA polymerase/promoter system represents a powerful system for expression of genes.

T7 RNA polymerase is a single polypeptide of molecular weight 98.8 kDa. The enzyme is specific for its own promoter, a conserved 23 bp sequence. *E. coli* DNA does not have promoter sequences for T7 RNA polymerase. The host RNA polymerase can be inhibited by the addition of rifampicin. Since *E. coli* mRNA decays rapidly, all mRNA in the cell is produced from the T7 RNA polymerase promoter. T7 RNA polymerase synthesizes RNA by a rapid and processive mechanism resulting in transcripts several times the plasmid length (Tabor and Richardson, 1985). The expression vector, pT7-7, has the promoter and the translational initiation region from bacteriophage T7. The *RbsD* gene has been placed under the control of the T7 transcription and translation elements. Upon induction the expression level of the *RbsD* gene product reaches that of the most abundantly expressed proteins in the cells. This high level of expression was exploited to develop a simple one-step purification protocol of the *RbsD* gene product.

The abundance of a protein in a cell has a direct bearing on the nature and number of steps required to purify the protein. Even when overexpression is obtained, purification protocols usually require some type of enrichment step such as ammonium sulfate precipitation, and a combination of chromatographic steps such

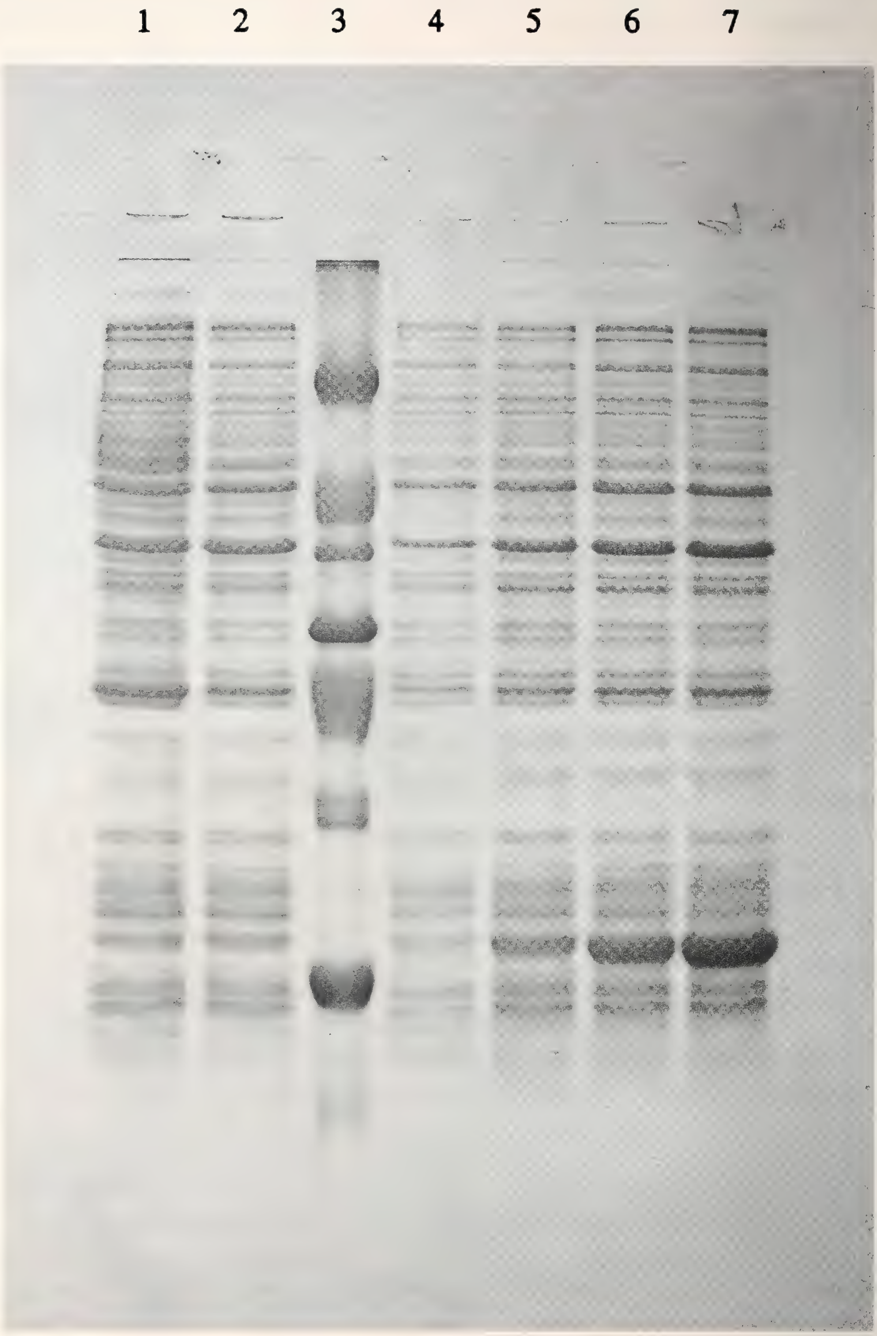


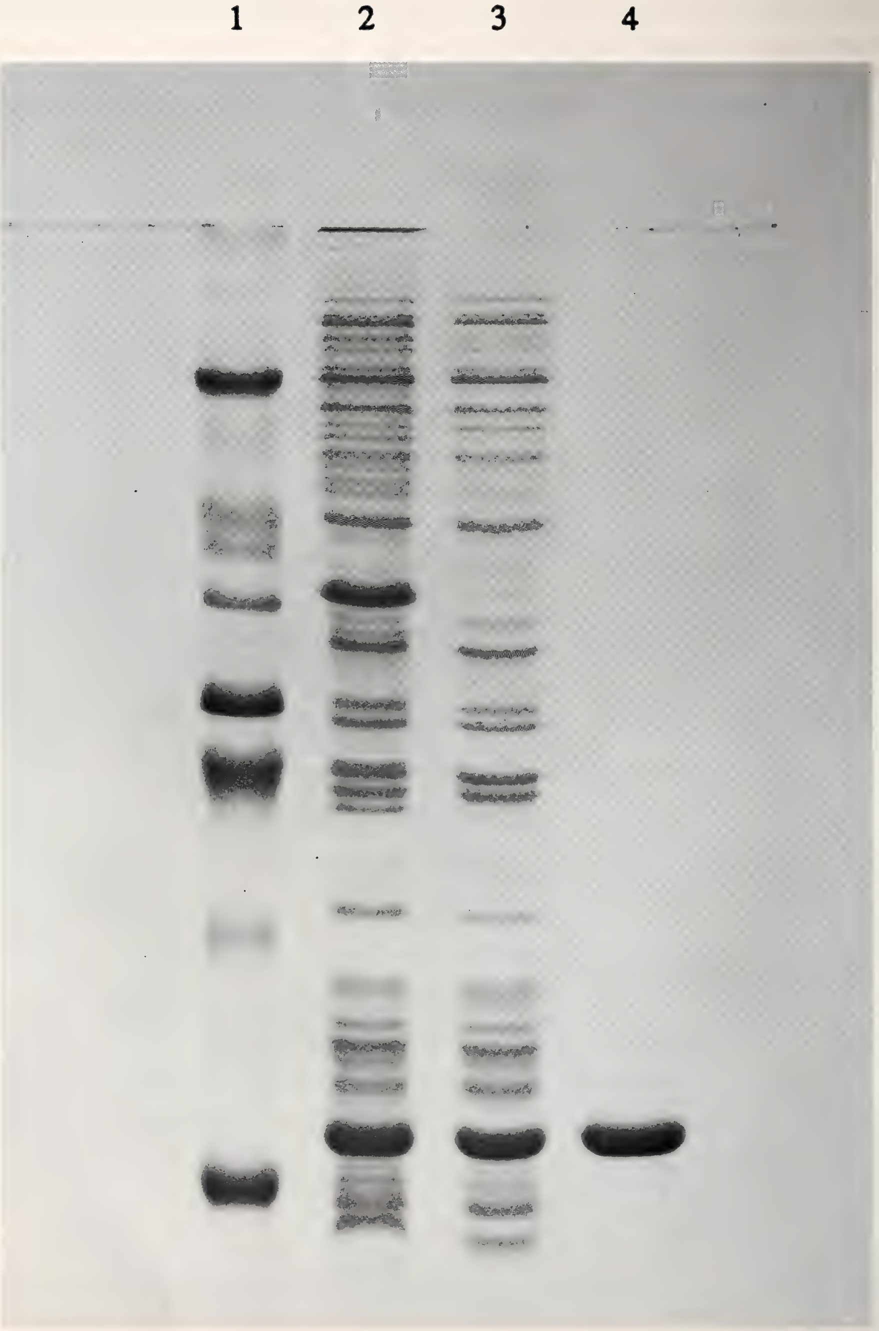
FIG. 1. Time course of RbsD expression. BL21(DE3)pLysS was transformed with pT7-7 and pT7D. Lanes 1 and 2 are samples from BL21(DE3)pLysS/pT7-7 uninduced and induced, respectively. Lane 3 shows molecular weight markers : 67 kDa, 45 kDa, 36 kDa, 29 kDa, 24 kDa, 20 kDa and 14 kDa. Lanes 4 through 7 are samples from BL21(DE3)pLysS/pT7D uninduced, induced 1, 2 and 3 hours respectively. The induced band at 15 kDa is RbsD. The samples were boiled 5 minutes before running on a 15% SDS polyacrylamide gel.

as ion exchange and size exclusion chromatography. Alternatively, a protein may be expressed as a fusion and purified using affinity chromatography based on the specific interaction of a fusion tag, *e.g.* His tag. But the presence of the tag may present a problem in characterization of the protein and require digestion and removal. The protocol presented here is a combination of high level expression and a natural affinity between RbsD and a hydrophobic matrix.

METHODS—The RbsD expression vector (pT7D) was constructed as follows. An EcoRI/HindIII fragment (containing *RbsD*) was cloned into the EcoRI/HindIII site of M13mp18 to yield M13mpRbsD. Single-stranded DNA was prepared for mutagenesis according to the Amersham oligonucleotide directed mutagenesis system version 2. An NdeI site was created at the initiation codon in M13mp18RbsD to give M13mp18RbsD*. The rbsD gene was excised as a DdeI/Klenow-NdeI fragment (*RbsD*) and cloned into the SmaI-NdeI site of pT7-7 to yield pT7D. The construction of pT7D was confirmed by DNA sequencing.

Plasmid-bearing strain BL21(DE3)/pLysS (pT7D) was inoculated into 1L of LB medium containing ampicillin (.1 mg/mL) and chloramphenicol (.05 mg/mL) and incubated with shaking at 37 °C. When the A_{600} reached 1, IPTG was added to a final concentration of 0.5 mM. After incubation at 37 °C for up to 3 hours, cells were harvested by centrifugation. All subsequent steps were performed at 4 °C. The pellet was resuspended in 20 mL of buffer A (100 mM ammonium bicarbonate, pH 7.8, 10% glycerol) containing 1 mM phenyl methyl sulfonyl fluoride (PMSF). Cells were broken by two passages in a French pressure cell at 12,000 psi. A clarified extract was obtained by centrifugation at 15,000 RPM for 15 minutes. The membranes were pelleted by centrifugation at 45,000 RPM for 6 hours. The membrane pellet was resuspended in 10 mL of buffer A. An equal volume of buffer A containing 20 mM EDTA was added and stirred for 30 minutes. The membranes were pelleted by centrifugation at 45,000 RPM for 6 hours. The supernatant was applied to a phenyl sepharose column that had been equilibrated with buffer A, and the proteins were eluted with buffer A. Fractions containing pure RbsD were pooled based on analysis by SDS-PAGE. The sequence of the N-terminal 6 amino acids of the purified protein was determined by automated Edman degradation on an Applied Biosystems 470A protein sequencer.

RESULTS AND DISCUSSION—RbsD was overexpressed in *E. coli* using the T7 expression system (Studier, 1991). An NdeI site was created at the initiation codon, and a DdeI/Klenow-NdeI fragment containing the coding region was subcloned in to the SmaI-NdeI site of pT7-7 to yield pT7D. This construct placed the *rbsD* gene under the transcriptional and translational controls of the phi 10 gene of the bacteriophage T7 (Tabor and Richardson, 1985). The host strain BL21(DE3)pLysS (Studier and Moffatt, 1986), supplies the T7 RNA polymerase and has additional advantages as well. The lysogen BL21(DE3) contains a single copy of the gene for T7 polymerase in the chromosome under the control of the inducible lac UV5 promoter. Some T7 RNA polymerase is produced from the prophage even in the absence of added inducer (IPTG), so plasmids containing target genes that are toxic can be difficult or impossible to maintain in BL21(DE3). To circumvent the problem of plasmid instability due to expression of toxic genes, bacteriophage T7 lysozyme is supplied to the host cells from plasmid pACYC184 (Studier, 1991). T7 lysozyme is a natural inhibitor of T7 RNA polymerase. T7 lysozyme mRNA is transcribed from the tet promoter in plasmid pACYC184. The lysozyme binds to and inhibits the T7 RNA polymerase, thus eliminating the basal activity from the inducible gene for T7 RNA polymerase. BL21 has the potential advantage that, as a B strain, it should be deficient in the *Lon* protease, and it lacks the *ompT* outer membrane protease that can degrade proteins during purification.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A time course study (Fig. 1) of RbsD production showed that RbsD becomes the most abundant protein 3 hours after induction. Plasmids pT7-7 (no insert) and pT7D were transformed into competent BL21(DE3)/pLysS cells, and synthesis of RbsD was induced by the addition of IPTG. Substantial overproduction of a protein of 15 kDa, which corresponds to the calculated molecular weight of the *rbsD* gene product (Bell et al., 1986), was observed. The cells containing overexpressed RbsD were fractionated into the membrane and soluble fractions, and the fractions were checked by SDS-PAGE to locate RbsD (Fig. 2). The bulk of the protein was located in the membrane fraction. However, most of the RbsD could be solubilized by washing the membranes with 10 mM EDTA. Therefore, membranes were prepared, extracted with 10 mM EDTA and the resulting soluble fraction subjected to hydrophobic interaction chromatography on a phenyl sepharose column (Fig. 2). The column was eluted with 100 mM ammonium bicarbonate containing 10% glycerol. Two peaks as measured by A_{280} readings emerged from the column. The second peak consisted of pure RbsD. To confirm that the purified protein was actually RbsD, the N-terminal 6 residues were determined by automated protein sequencing using the Edman degradation method. The sequence obtained (MetLysLysGlyThrVal) agrees perfectly with the deduced amino acid sequence from the *RbsD* gene. No evidence for heterogeneity was observed from the sequence data. The C-terminal region of RbsD is hydrophobic and may be responsible for the affinity between the protein and the column. This simple one-step protocol represents quite an improvement of a protocol used by Kim and coworkers (2001). In their procedure the cellular extract was subjected to ammonium sulfate precipitation followed by resuspension and dialysis. Afterwards, two columns were used to purify the protein. First an ion exchange column was run and then a size exclusion column. The procedure presented here calls for the cellular extract to be applied directly to a hydrophobic interaction column, and pure RbsD is obtained from the column. This protocol is simple, fast and very efficient in producing pure RbsD and should facilitate production of large amounts of pure RbsD for detailed characterization.

ACKNOWLEDGMENTS—The author wishes to give special thanks to the National Institutes of General Medical Sciences for support of this work through grant Number SO6 GM 08111.

LITERATURE CITED

BELL, A., S. BUCKEL, J. GROAKE, J. HOPE, D. KINGSLEY, AND M. HERMODSON. 1986. The nucleotide sequences of the *rbsD, rbsA* and *rbsC* genes of *E. coli* K12. J. Bio. Chem. 261:7652–7658.

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FIG. 2. Purification of RbsD. Strain BL21(DE3)pLysS/pT7D was grown in LB with ampicillin and chloramphenicol. Membranes were prepared from induced cells. The membranes were washed with 10 mM EDTA to release RbsD. The solubilized EDTA extract was applied to a phenyl sepharose column which had been equilibrated with 100 mM ammonium bicarbonate buffer, pH 7.8 containing 10% glycerol. The column was eluted with the same buffer. Samples from the purification were subjected to electrophoresis on 15% SDS polyacrylamide gel. Lane 1, molecular weight markers; lane 2, crude membrane fraction; lane 3, EDTA solubilized fraction; lane 4, pure RbsD.

KIM, M., H. OH, C. PARK, AND B. OH. 2001. Crystallization and preliminary X-ray crystallographic analysis of Escherichia coli RbsD. Acta. Crystallographica Section D. 57:728–730.

STUDIER, F. 1991. Use of bacteriophage T7 lysozyme to improve an inducible T7 expression system. J. Mol. Biol. 219:37–44.

——— AND B. MOFFATT. 1986. Use of bacteriophage T7 RNA polymerase to direct selective high level expression of cloned genes. J. Mol. Biol. 189:113–130.

TABOR, S. AND C. RICHARDSON. 1985. A bacteriophage T7 RNA polymerase and promoter system for controlled exclusive expression of specific genes. Proc. Natl. Acad. Sci. USA. 82:1074–1078.

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RbsD OF THE D-RIBOSE HIGH-AFFINITY TRANSPORT SYSTEM OF *ESCHERICHIA COLI* IS NOT AN OUTER MEMBRANE RECEPTOR

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Abstract: *The D-ribose high-affinity transport operon (RbsDACBKR) of* Escherichia coli *encodes the proteins necessary for high-affinity transport and utilization of D-ribose. RbsD is a 15 kDa protein with an amino terminus which resembles the proteolytically cleaved signal sequences of periplasmic and outer membrane proteins. N-terminal sequencing of the purified protein revealed that the protein does not contain a cleavable signal sequence and is therefore not likely to be an outer membrane receptor. Characterization of a Rbs operon mutant at the RbsD site reveals that D-ribose high-affinity transport does not require RbsD.*

Key Words: RbsD, D-ribose transport

Genes required for the utilization of D-ribose (*Rbs*) as a carbon source by Escherichia coli K12 have been mapped to 84 minutes on the E. coli chromosome (Anderson and Cooper, 1970). Genetics studies revealed a D-ribose high-affinity transport operon (Lopilato et al., 1984; Iida et al., 1984) and established the order of genes as *RbsP/OrbsACBK*. *RbsA* and *RbsC* encode proteins involved in D-ribose high-affinity transport, *RbsB* encodes D-ribose binding protein, also required for transport, and rbsK encodes ribokinase. The existence of a second low-affinity transport system was also demonstrated. The *Rbs* high-affinity transport operon was shown to be subject to negative control by the *RbsR* gene product, which was thought to be expressed from a separate transcriptional unit. Operon sequence analysis (Bell et al., 1986; Hope et al., 1986) of the D-ribose high-affinity transport operon identified an additional gene, *RbsD*, and redefined the order as *RbsDACBKR*.

Although the arrangement of the *Rbs* operon would suggest that the *RbsD* gene product is part of the transport complex along with the *RbsA* and *RbsC* gene products, no evidence exists which would implicate *RbsD* as an essential gene for ribose transport. Also, the arabinose and galactose (Scripture and Hogg, 1987) transport systems, the two most similar to *Rbs*, have no *RbsD* counterpart. The *RbsD* gene product does not share homology with any component of other binding protein dependent transport systems (Bell et al., 1986), and it possesses two unique characteristics. First, it is smaller (15 kDa) than all other components of binding protein dependent transport systems, the next smallest being about 25 kDa (Ames, 1986). Second, the amino terminal 20 residues have some of the characteristics of the signal sequences of periplasmic and outer membrane proteins, suggesting that the *RbsD* gene product may be exported.

To determine if the *RbsD* gene is required for D-ribose high-affinity transport, a *Rbs* operon mutant was constructed. The mutant contains a deletion of the amino terminal half of the *RbsD* gene product.

METHODS—The mutant was made using the Amersham Oligonucleotide-directed *in vivo* mutagenesis system, version 2. Transport assays were performed according to Iida and coworkers (1984) as follows. Cells were grown in M9 minimal medium containing 0.4% glycerol, 0.2% ribose and a supplement of trace elements. The cells were washed four times with 10 mM KPO_4 (pH 6.9) at 0°C and suspended to an optical density at 660 nm of 0.3 (about 3×10^8 cells per mL) in 0.1 M KPO_4 (pH 7.2). A 0.1 mL sample of chloramphenicol (0.4 mg/mL) was added to 0.8 mL of the cell suspension and the mixture was brought to 30°C. A 0.1 mL sample of [^{14}C]ribose was added at 0 time. At 15 or 30 s, 0.8 mL of the mixture was filtered on a 1225 Sampling Manifold (Millipore Corporation, Bedford, Mass.) and washed with 5 mL of a buffer consisting of 10 mM Tris (pH 7.3), 0.5 mM $MgCl_2$, 0.15 M NaCl at 0°C. The filters were dried and the radioactivity was determined by liquid scintillation counting. Values for nonspecific binding of ribose on the cells and on the filter were estimated by using cells treated with 10% formaldehyde.

RESULTS AND DISCUSSION—The deduced amino acid sequence (Bell et al., 1986) of RbsD contains an amino terminal region which has some features of the leader (signal) sequences of exported proteins. However, the charge properties of the RbsD amino terminus are different from those of the well characterized signal sequences (Heijne, 1985). Cleavable signal sequences of the periplasmic proteins have the characteristic features necessary for export: positive charges at the amino terminus followed by a long stretch of hydrophobic residues and a cleavable site for signal peptidase.

Uncleaved signal-like sequences have quite different charge properties. They may not have positive charges located at the amino terminus. They usually have charged groups located within the hydrophobic stretch of residues. The RbsD sequence does have the positive charges at the amino terminus, but it also has other charged residues interspersed throughout the hydrophobic stretch of amino acids: Asp at position 10, Arg at position 17 and His at position 20.

RbsD is much smaller than outer membrane receptors. But there are cases of small proteins, such as Lom (Reeve and Shaw, 1979) and Ail (Miller et al., 1990) being located in the outer membrane. To determine if RbsD does indeed possess a cleavable signal, the purified protein was subjected to six cycles of Edman degradation on an automatic protein sequencer. The sequence obtained (MetLysLysGlyThrVal) agrees exactly with the deduced amino acid sequence from the *RbsD* gene. No evidence for heterogeneity was observed from the sequence data. Futhermore, the protein always migrated on SDS gels at a position relative to the molecular weight corresponding to 15 kDa, which would indicate that RbsD did not undergo processing. All proteins known to be localized in the outer membrane are synthesized with a cleavable signal sequence. RbsD does not have a cleavable signal sequence and is not likely to be an outer membrane receptor. It is very likely though that the RbsD amino terminus is capable of exporting the protein from the cytoplasm. A RbsD-Alkaline Phosphatase fusion at the 18th residue of RbsD has been constructed (Barroga et al., 1996). It has high alkaline phosphatase activity indicating a periplasmic location. This fusion protein can be released from the periplasm by chloroform shock treatment which

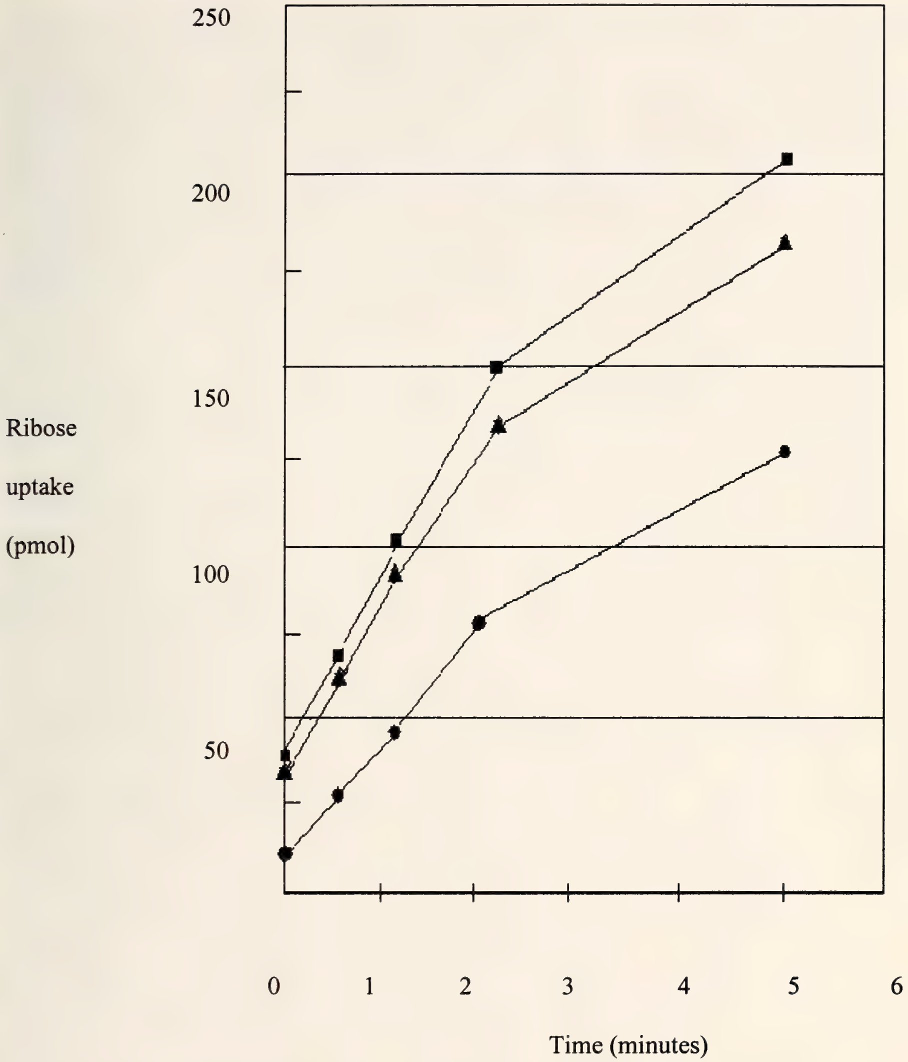

FIG. 1. Kinetics of ribose uptake. Squares represent the wild-type strain; Triangles represent the *tonB* deletion strain; Circles represent the strain with the N-terminal RbsD deletion.

releases the soluble periplasmic ribose binding protein. This implies that the N-terminal 18 residues are capable of exporting the fusion protein from the cytoplasm. This has been observed in several other cases, namely, TonB (Roof et al., 1991), FecR (Hove et al., 1990) and BtuE (Friedrich et al., 1986). These proteins have signal-like sequences similar to RbsD. They also have additional charge groups in their sequences, and they have no recognition site for signal peptidase.

To examine the involvement of RbsD in D-ribose high-affinity transport, a *rbs* operon mutant was constructed. The mutant had the amino terminal half of RbsD deleted. Figure 1 shows the results from the transport assays. The RbsD deletion mutant showed a large decrease in D-ribose high-affinity transport. But it is likely that transport can take place without RbsD, since the deletion mutant can transport 60% as much ribose as the control. The decrease in transport can be interpreted primarily as a rate effect. The Vmax of the deletion mutant is reduced 7-fold. The genetic data presented here indicate that RbsD is not essential for D-ribose high-affinity transport. Kinetic results analogous to the data presented here have been reported for the *mgl* system. Ordal and Adler (1974) found that mutations in *MglA* and *MglC* shifted the Km for transport to lower concentrations and caused moderate to severe decreases in Vmax. The deletion mutant was capable of transporting 60% as much ribose as the control, indicating that RbsA and RbsC are capable of transporting ribose in the absence of RbsD. These results are not shocking when one considers the other periplasmic permeases which are most similar to the *Rbs* system, *Ara* and *Mgl* (Scripture et al., 1987). Both these systems have the required periplasmic binding protein, a very hydrophobic protein capable of forming a pore or channel in the membrane (AraH and MglC), and a protein with the consensus nucleotide binding site which has been implicated in ATP hydrolysis (AraG and MglA). Both of these proteins and RbsA have N-terminal and C-terminal halves which are homologous to each other. Thus, without RbsD, the *Rbs* operon appears almost exactly like the *Ara* and *Mgl* operons with respect to genetic organization. Futhermore, the deduced sequences of the components for the *Ara, Mgl* and *Rbs* systems indicate high homology among the proteins. The AraG and RbsA proteins share 43% identity. AraH and RbsC share 36% identity. The MglA and RbsA proteins share 45% identity and 67% similarity. MglC and RbsC share 38% identity and 64% similarity. Thus the transport proteins are very similar as are the substrates that are transported. So it seems likely that it is the periplasmic binding protein (RbsB), the hydrophobic membrane pore or channel (RbsC) and the ATPase component (RbsA) that form the essential components for high-affinity transport. It appears that there is a subset of periplasmic permeases which require only two components for the formation of a viable membrane associated transport complex. The arabinose system has been sequenced and reconstituted genetically (Horazdovsky and Hogg, 1989), and it has been shown that only AraG and AraH are necessary in addition to the AraF binding protein for transport of arabinose. Likewise the *Mgl* system has been sequenced, and only MglA and MglC appear to be necessary in addition to the MglB binding protein for the transport of galactose. The evidence presented here indicates that only RbsA, RbsC and the RbsB binding protein are necessary for the high-affinity transport of D-ribose.

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LITERATURE CITED

AMES, G. 1986. Bacterial periplasmic transport systems: structure, mechanism and evolution. Ann. Rev. Biochem. 55:397–425.

ANDERSON, A. AND R. COOPER. 1970. Biochemical and genetical studies on ribose catabolism in *E. coli* K12. J. Gen. Microbiol. 62:335–339.

Barroga, C., H. Zhang, N. Wajih, J. Bouyer, and M. Hermodson. 1996. The proteins encoded by the *Rbs* operon of *E. coli*: I. Overproduction, purification, characterization, and functional analysis of RbsA. Protein Science. 5:1093–1099.

Bell, A., S. Buckel, J. Groake, J. Hope, D. Kingsley, and M. Hermodson. 1986. The nucleotide sequence of the *RbsD, RbsA* and *RbsC* genes of *E. coli* K12. J. Bio. Chem. 261:7652–7658.

Friedrich, M., L. Veaux, and R. Kadner. 1986. Nucleotide sequence of the *BtuCED* genes involved in vitamin B12 transport. J. Bacteriol. 167:928–934.

Heijne, G. 1985. Signal sequences: The limits of variation. J. Mol. Biol. 184:99–105.

Hope, J., A. Bell, M. Hermodson, and J. Groake. 1986. Ribokinase from *E. coli* K12. J. Biol. Chem. 261:7663–7668.

Horazdovsky, B. and R. Hogg. 1989. Genetic reconstitution of the high-affinity L-arabinose transport system. J. Bacteriol. 171:3053–3059.

Hove, B., H. Staudenmaier, and V. Braun. 1990. Novel two-component transmembrane transcription control: Regulation of iron dicitrate transport in *E. coli* K12. J. Bacteriol. 172:6749–6758.

Iida, A., S. Harayama, T. Iino, and G. Hazelbauer. 1984. Molecular cloning and characterization of genes required for ribose transport and utilization in *E. coli* K12. J. Bacteriol. 158:674–682.

Lopilato, J., J. Garwin, S. Emr, T. Silhavy, and J. Beckwith. 1984. D-ribose metabolism in *E. coli* K12: Genetics, regulation and transport. J. Bacteriol. 158:665–673.

Miller, V., J. Bliska and S. Falkow. 1990. Nucleotide sequence of the Yersinia enterocolitica *Ail* gene and characterization of the Ail product. J. Bacteriol. 172:1062–1069.

Ordal, G. and J. Adler. 1974. Properties of mutants of galactose taxis and transport. J. Bacteriol. 117:517–526.

Reeve, J. and J. Shaw. 1979. Lambda encodes an outer membrane protein: The *Lom* gene. Molec. Gen. Genet. 172:243–248.

Roof, S., J. Allard, K. Bertrand, and K. Postle. 1991. Analysis of *E. coli* TonB membrane topology by use of PhoA fusions. J. Bacteriol. 173:5554–5557.

Scripture, J. and R. Hogg. 1987. High-affinity L-arabinose transport operon. J. Mol. Biol. 197:37–46.

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IMPLICATION OF RbsD OF THE D-RIBOSE HIGH-AFFINITY TRANSPORT SYSTEM OF *ESCHERICHIA COLI* AS A REGULATOR OF RIBOKINASE ACTIVITY

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Abstract: *The D-ribose high-affinity transport operon (RbsDACBKR) of* Escherichia coli *encodes the proteins necessary for high-affinity transport and utilization of D-ribose. rbsD encodes a 15 kDa protein that is not essential for D-ribose high-affinity transport. RbsD is unique as a component of a high-affinity transport system in that it has a TonB box at its amino terminus. Neither RbsD or RbsK are required for D-ribose high-affinity transport, but their potential interaction with each other has not been examined. Characterization of a rbs operon mutant at the TonB box of RbsD implicates the RbsD gene product as a regulator of ribokinase (RbsK) activity.*

Key Words: RbsD, TonB, RbsK

The gram-negative bacterium *Escherichia coli* can utilize D-ribose as the sole source of carbon and energy. There are two permeases for D-ribose in E. coli (Lopilato et al., 1984; Iida et al., 1984). One is an uncharacterized low-affinity D-ribose permease. The other is a high-affinity D-ribose binding protein dependent (periplasmic) permease. The DNA sequence of the D-ribose high-affinity transport operon (*rbs*) has been determined (Bell et al., 1986) and six structural genes have been identified, *rbsP/OrbsDACBKR*. Genetic studies had previously revealed that *RbsA* encodes a 50 kDa protein required for high-affinity transport, *RbsC* encodes a 27 kDa protein involved in high-affinity transport, *RbsB* encodes the 29 kDa periplasmic ribose binding protein, which is also required for transport, and *RbsK* encodes the 34 kDa ribokinase. The *Rbs* operon is subject to negative control by the *RbsR* gene product.

The function of *RbsD* remains an enigma. D-ribose transport can take place in the absence of RbsD. Therefore, RbsD is not a constituent of the high-affinity transport complex. The RbsD protein contains the pentapeptide sequence AspThrLeuValVal at residues 22–26. A similar sequence is present in a similar position in the sequence of outer membrane receptor proteins which are constituents of TonB dependent transport systems and in all colicins which are taken up by a TonB dependent mechanism (Postle, 1990).

To determine if the *RbsD* gene is required for D-ribose utilization, a *Rbs* operon mutant was constructed. The mutant has an alteration of the putative TonB box. A mutant strain with a *TonB* deletion in its chromosome, but wild-type *Rbs* operon on its chromosome was also examined.

METHODS—The mutant was made using the Amersham Oligonucleotide-directed in vivo mutagenesis system, version 2. Ribokinase assays were according to Lopilato and coworkers (1984) as follows. Cells were grown to the midlog phase in LB medium, washed, concentrated 10-fold in 10 mM Tris-hydrochloride (pH 7.4), and sonicated for 20 s four times with 1-min intervals between each sonication. After sonication, the extracts were centrifuged at 6,000 rpm to remove unbroken cells. The supernatant was used to determine the amount of ribokinase activity by the method of Sable (1955). Assays were done at room temperature. Ribose was converted to ribose-5-phosphate, and the amount of ribose remaining was determined by using the method of Nelson (1944).

RESULTS AND DISCUSSION—The deduced amino acid sequence (Bell et al., 1986) of RbsD contains an amino terminal region which has some features of the leader (signal) sequences of exported proteins. Following the signal-like sequence is the pentapeptide AspThrLeuValVal. This pentapeptide is present near the amino termini in all TonB dependent outer membrane receptors. These receptors depend upon TonB for transport of molecules across the outer membrane. Although most of these proteins are involved in ligand transport, these receptors share some general features: (1) a cleavable signal sequence which targets them for the outer membrane, (2) the TonB box located near the amino terminus of the mature protein, and (3) a region in the amino terminal half which localizes the protein in the outer membrane (Nau and Konisky, 1989). This overall structure is present in RbsD. The sequence of the first 20 residues resembles that of a signal peptide, except that extra charged residues are present. Following the signal-like sequence is the TonB box located at residues 22–26. And there is a stretch of hydrophobic residues, 42–61 (Bell et al., 1986), which could serve as a membrane attachment site. Previous studies demonstrated that RbsD does not have a cleavable N-terminal signal sequence and therefore is not likely to be an outer membrane receptor. The protein may however be localized to the inner membrane with part of the protein exposed to the periplasm. Previous studies also demonstrated that RbsD is not required for D-ribose high-affinity transport. The genetic makeup of the *Rbs* operon (*RbsDACBKR*) establishes the possibility that there may be some interaction between RbsD and one of the other nontransport components-RbsK or RbsR.

To examine the involvement of RbsD in D-ribose utilization (interaction with RbsK), a *Rbs* operon mutant was constructed. The mutant had the TonB box altered. The sites for the TonB box mutation were based on previous results (Kadner, 1990) which indicated that it is local secondary structure and not specific residues which is important for interaction with TonB. Ribokinase assays were performed on this *Rbs* operon mutant and a mutant strain with a *TonB* deletion in the chromosome, but carrying wild-type *rbs* operon on the chromosome.

The results from the ribokinase assays (Table 1) are very interesting. The TonB box mutant exhibited about 30% more ribokinase activity than the control. The strain with the chromosomal deletion of *TonB* had about twice as much ribokinase activity as its wild-type counterpart. Taken together, these data imply that RbsD and TonB are both involved in D-ribose utilization. Superficially, RbsD resembles a TonB dependent outer membrane receptor. TonB functions to transduce energy between the inner and outer membranes (Postle, 1990). Specifically, TonB couples the electrochemical potential of the cytoplasmic membrane to the active transport of

TABLE 1. Phenotype of *Rbs* strains.

Strain	Mutation	Ribokinase activity[a], %
Sample 1	Control None	100
Sample 2	TonB Box (L24P, V25A, V26G)	135 ± 6.75
Sample 3	*TonB* deletion	150 ± 7.25

[a] Expressed as the relative percentage of the control.

vitamin B12 and ferric siderophores across the outer membrane, to the energy-dependent steps of bacteriophage phi 80 and T1 infection, and to the entry of group B colicins into bacteria. D-ribose is a small (150 Da) hydrophilic sugar. The outer membrane allows passage of small (<600 Da) hydrophilic molecules into the periplasm by simple diffusion through nonspecific pores (Nikaido and Vaara, 1985). Therefore, it is unlikely that a component of the D-ribose high-affinity transport system would function as a TonB dependent outer membrane receptor.

The uniqueness of the *Rbs* operon system with repect to the *ara* and *mgl* is the presence of additional genes, namely *RbsD, RbsK* and *RbsR*. The data presented here introduce a phenomenological relationship between RbsD, TonB and RbsK. If RbsD and TonB do interact, and if this interaction plays a role in ribokinase expression and/or activity, this would present a new role for TonB. Our data showed a 35% increase in ribokinase activity for the *TonB* box mutant. This is not a very large increase, but the fact that this mutant showed an increase in ribokinase activity is interesting. Furthermore, the *TonB* deletion strain showed 50% more ribokinase activity than the otherwise isogenic wild-type strain. These data suggest that RbsD might be involved in regulation of ribokinase, and that the RbsD/TonB interaction represses ribokinase expression. Then once the RbsD/TonB interaction is broken, ribokinase expression increases. It seems reasonable that there would be a connection between *RbsD* and *RbsK*. Neither one is necessary for transport, so the presence of *RbsD* on the *Rbs* operon may be due to similar factors which dictate that *RbsK* be part of the operon.

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LITERATURE CITED

BELL, A., S. BUCKEL, J. GROAKE, J. HOPE, D. KINGSLEY, AND M. HERMODSON. 1986. The nucleotide sequence of the *rbsD, rbsA* and *rbsC* genes of *E. coli* K12. J. Bio. Chem. 261:7652–7658.

IIDA, A., S. HARAYAMA, T. IINO, AND G. HAZELBAUER. 1984. Molecular cloning and characterization of genes required for ribose transport and utilization in *E. coli* K12. J. Bacteriol. 158:674–682.

KADNER, R. 1990. Vitamin B12 transport in *E. coli*: Energy coupling between membranes. Mol. Microbiol. 4:2027–2033.

LOPILATO, J., J. GARWIN, S. EMR, T. SILHAVY, AND J. BECKWITH. 1984. D-ribose metabolism in *E. coli* K12: Genetics, regulation and transport. J. Bacteriol. 158:665–673.

NAU, C. AND J. KONISKY. 1989. Evolutionary relationship between the TonB-dependent outer membrane transport proteins: nucleotide and amino acid sequences of the *E. coli* colicin I receptor gene. J. Bacteriol. 171:1041–1047.

NELSON, N. 1944. A photometric adaptation of the Somogyi method for the determination of glucose. J. Biol. Chem. 153:375–380.

NIKAIDO, H. AND M. VAARA. 1985. Molecular basis of bacterial outer membrane permeability. Microbiol. Rev. 49:1–32.

POSTLE, K. 1990. TonB and the gram-negative dilemma. Mol. Microbiol. 4:2019–2025.

SABLE, H. 1955. Yeast pentokinase. Methods Enzymol. 1:357–360.

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OBITUARY

Joseph L. Simon

3/16/1937–3/22/04

Joe Simon served as President of the Florida Academy of Sciences in 1980 and continued his support of the organization until he retired not long ago. He also served as Chair of the Division of Invertebrate Zoology of the American Society of Zoology in the late 1970s and was professionally active in the American Association for the Advancement of Science in addition to other professional activities.

He was born in Everett, Massachusetts, was graduated from Tufts University, and earned a doctorate in zoology from the University of New Hampshire (1963).

He joined the faculty of the University of South Florida in 1964 where he remained (apart from a leave of absence at Woods Hole in 1965 and serving as a Fulbright Scholar in Taiwan in 1989–90) until his retirement in 2003.

He was strongly interested in the ecology of Tampa Bay, and helped reintroduce sea grasses to that bay, and contributed significant information [Florida Scient. 37(4): 217–244]. He specialized in the study of polycheates and used their numbers and distribution to assess the environmental quality of the bay. This became particularly significant when a red tide invasion of Tampa Bay in 1971 appeared to kill most of the marine worms. Joe Simon obtained a special grant from NSF to study regeneration of the invertebrate fauna of the bay. He helped organize a major symposium on Tampa Bay, and co-edited a special book on the environmental status of the bay [Proceedings, Tampa Bay Area Scientific Information Symposium (Burgess, May 1984)].

Joe was among the number of faculty who contributed to the first Ph.D. program at USF (in Marine Biology). He was a stalwart in the early development of the program in marine science. He is said to have been the first USF faculty member to generate $1 million in sponsored research grants.

On the other hand, Joe Simon was a dedicated teacher who loved being in the classroom and challenging students to learn. He had a no-nonsense approach that must have been a joy to those interested in learning and a pain to those who weren't. His efforts were recognized in many ways, but perhaps most notably when his students organized a banquet (1994) to celebrate his 30th year in teaching at USF.—Dean F. Martin, University of South Florida.

[Information was provided by colleagues and by an obituary from the Tampa Tribune written by Mr. Gary Haber.]

REVIEW

Richard P. Wunderlin and Bruce F. Hansen, *Guide to the Vascular Plants of Florida*, Second Edition, Pp. 787. Hard Cover. Price $39.95.

ALTHOUGH the botanical literature of the state of Florida is quite extensive there has not been a single publication that treats the whole territory of Florida and all the taxa occurring there. Richard P. Wunderlin therefore breaks new ground by producing the first comprehensive, synoptic treatment for the flora of Florida, with more than 4,000 species of vascular plants. His work is scholarly in nature as he only treats taxa that are vouchered by specimens or that are cited in authoritative publications. Plant descriptions are limited to the keys, making this publication succinct enough to fit in a single volume that can easily be carried into the field. But do not let its small size fool you, it is equally valuable as a source of reference, as it contains keys to all taxonomic levels, extensive lists of synonyms and common names, and a glossary of technical terms. This is proven by the fact that this work has stimulated the interest in local flora, leading to new records and therefore to a second edition. The second edition which incorporates Bruce F. Hansen as coauthor, as expected, is as authoritative as the first edition. The format has been reworked to include about 100 additional taxa in a volume that contains 21 pages less than the first edition, and families are now presented in alphabetic order (vs. phylogenetic). Family concepts have been modified as well, in order to reflect the most current findings of plant systematics research, contributing this way to the diffusion of more natural family concepts to new generations of biologists. This volume sets the foundation for the ongoing multivolume *Flora of Florida* by the same authors, and makes you realize the value that such a treatment can give to multivolume floristic projects.—Pedro Acevedo-Rodríguez, Smithsonian Institution, Department of Botany, MRC-166, P.O. Box 37012, Washington, DC 20013.

REVIEW

Duncan C. Blanchard, *From Raindrops to Volcanoes,* Dover 2004 (reprint of 1967). xii+196 pp. Paper, $11.95 (ISBN:0-486-43487-7).

It was my personal pleasure to correspond with the author prior to his retirement in 1989. He was an internationally recognized expert as well as a gracious correspondent. He had become an expert in sea surface metallurgy, especially in the production and impact of droplets produced at the sea surface. His research did much to clarify the mysteries of concentrations in rainfall, and how the rain over certain parts of Florida could have phosphorus levels above those that some were trying to use as legal minimums. His research also was significant in understanding the impact of toxin-containing aerosols associated with Florida red tides. In this interesting account of his lifelong interest in meteorology, he describes his experiments and provides information on how they can be easily (and cheaply) reproduced. The book is packed with interesting and significant information, e.g., for example on why rain drops aren't tear-shaped as one might suspect, why the sea air is salty, the importance of jet drops from the sea, atmospheric electricity associated with volcanoes, and volcanoes and impacts on the ocean and atmosphere. The book is recommended for general-interest reading, for adult and younger readers, and there are undoubtedly ideas for future Junior Academy of Science projects lurking in this interesting book.—Dean F. Martin, University of South Florida, Tampa, FL.

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This information is available at two web sites:

(1) **IES site**: http:www.chuma1cas.usf.edu (click on "Centers and Institutes", then select "Institute for Environmental Studies", then select "Florida Scientist").

(2) **FAS site**: http://www.floridaacademyofsciences.org (select "Florida Scientist").

It is possible to search for papers and abstracts of papers for the past six years: Go the FAS site (above), select "Allen Press". When the link is complete, select "Advanced Search" and follow the directions.

The Florida Academy of Sciences Web page, indicated above, allows interested persons to join the Florida Academy of Sciences and pay dues.

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